

CONT. ON FIG.1b

FIG.1a

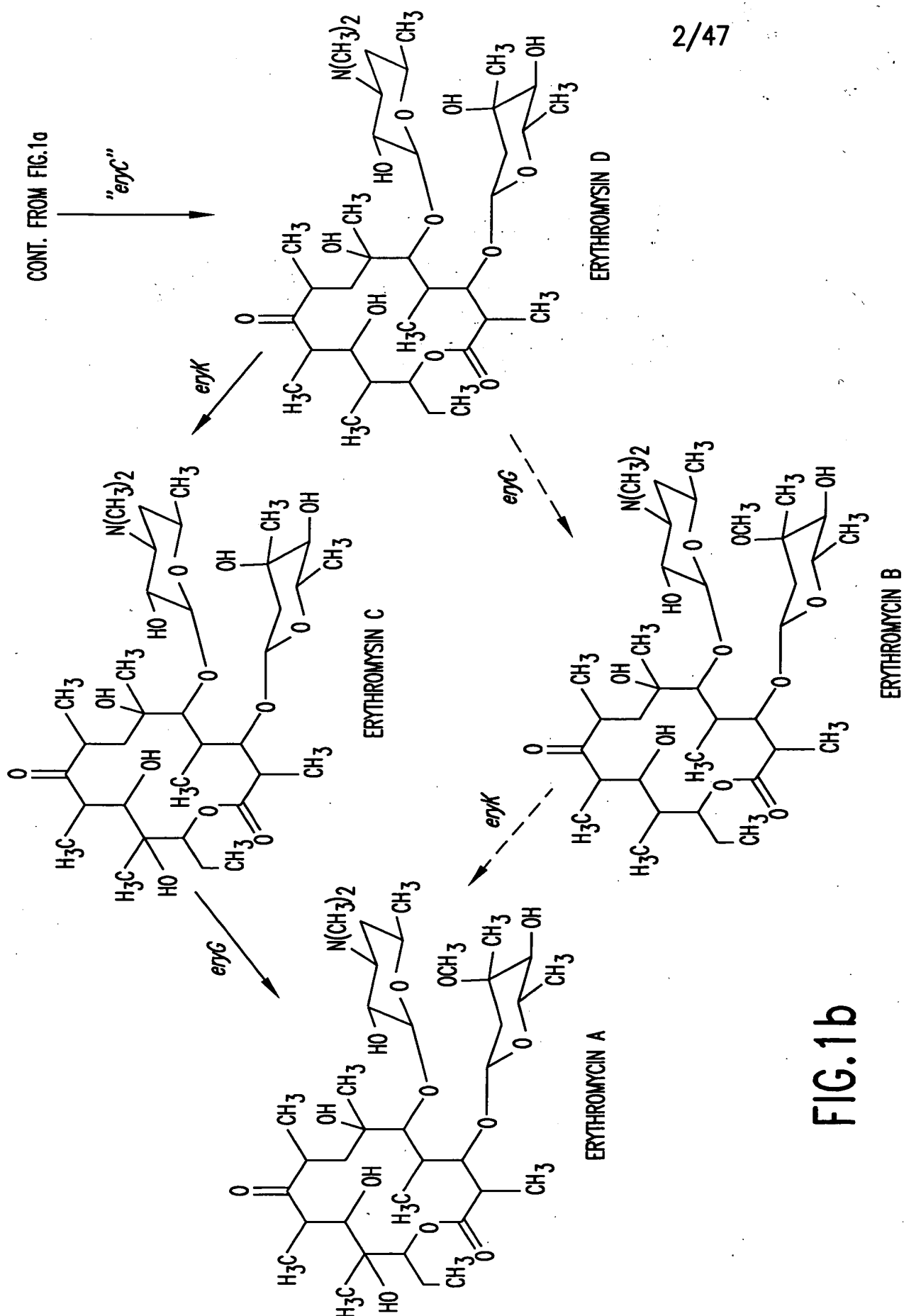


FIG.1b

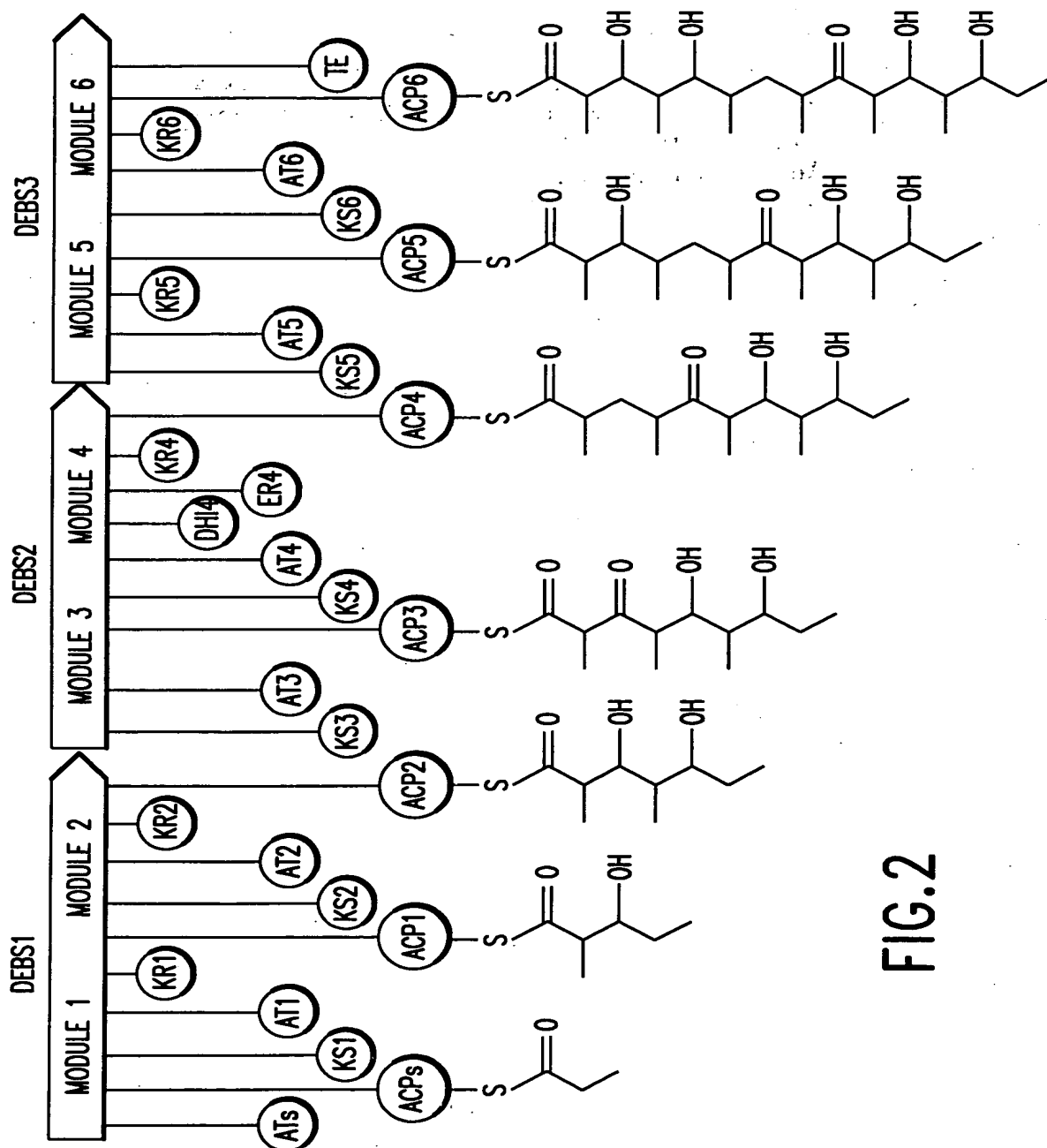


FIG.2

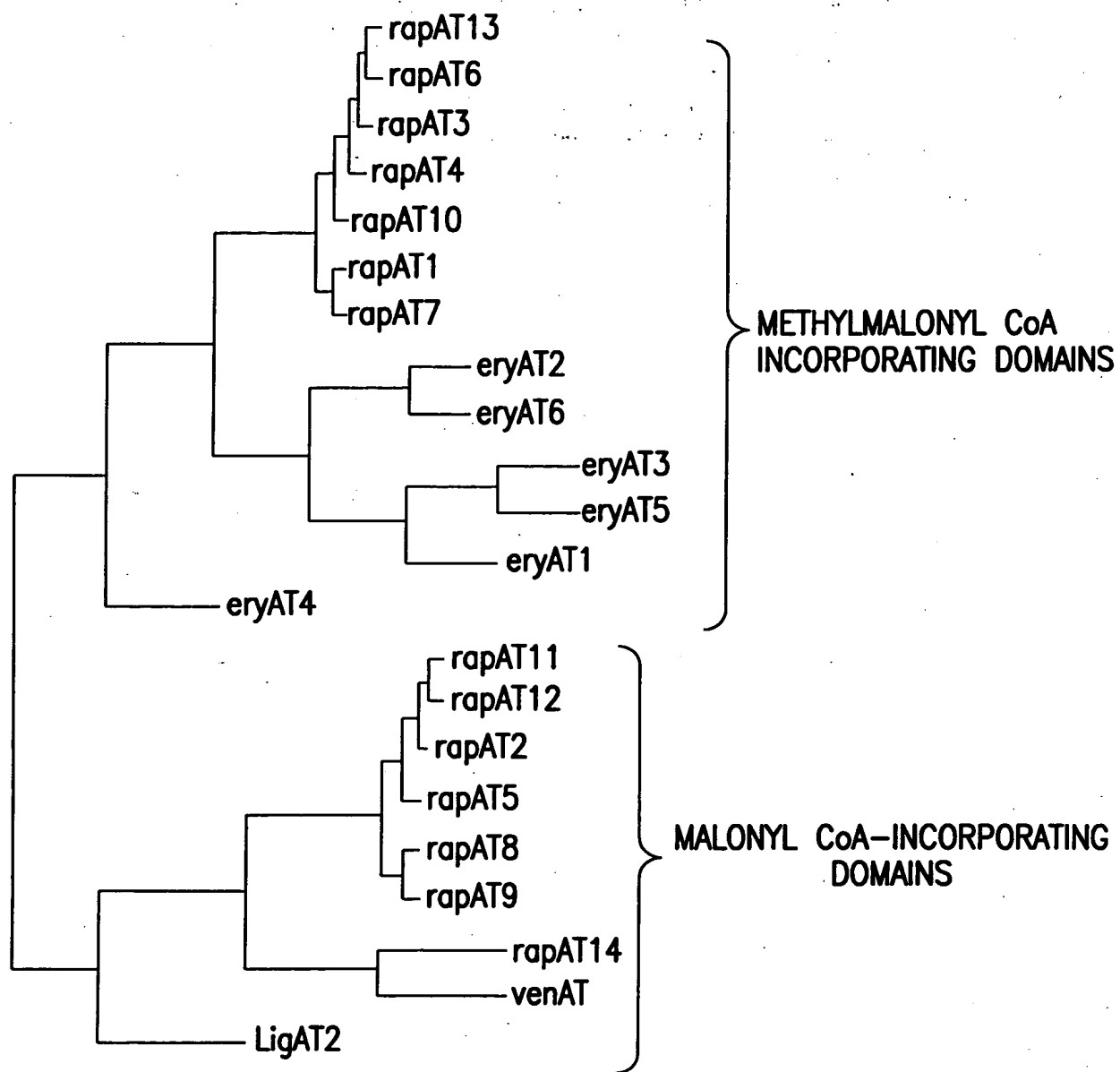
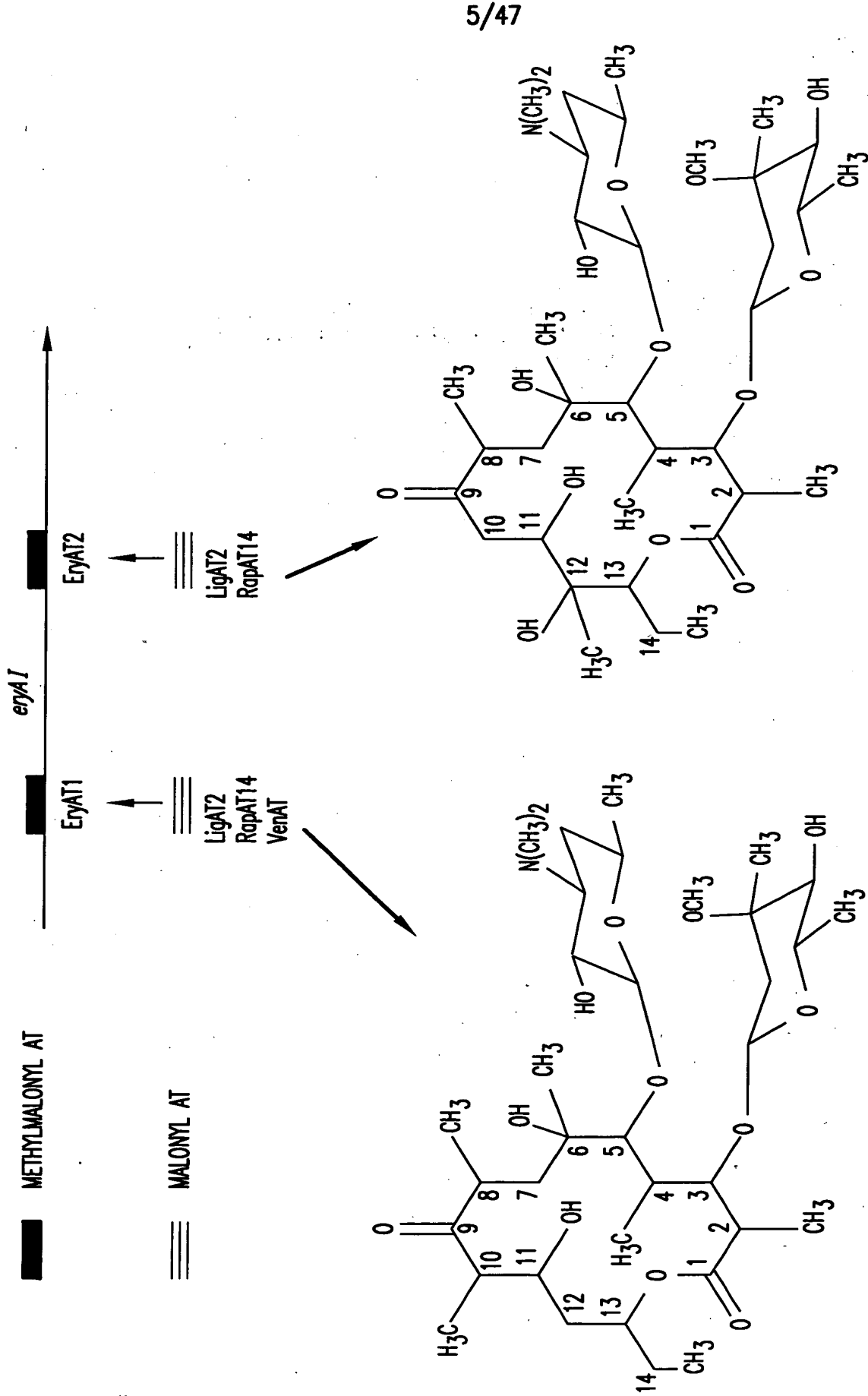


FIG.3





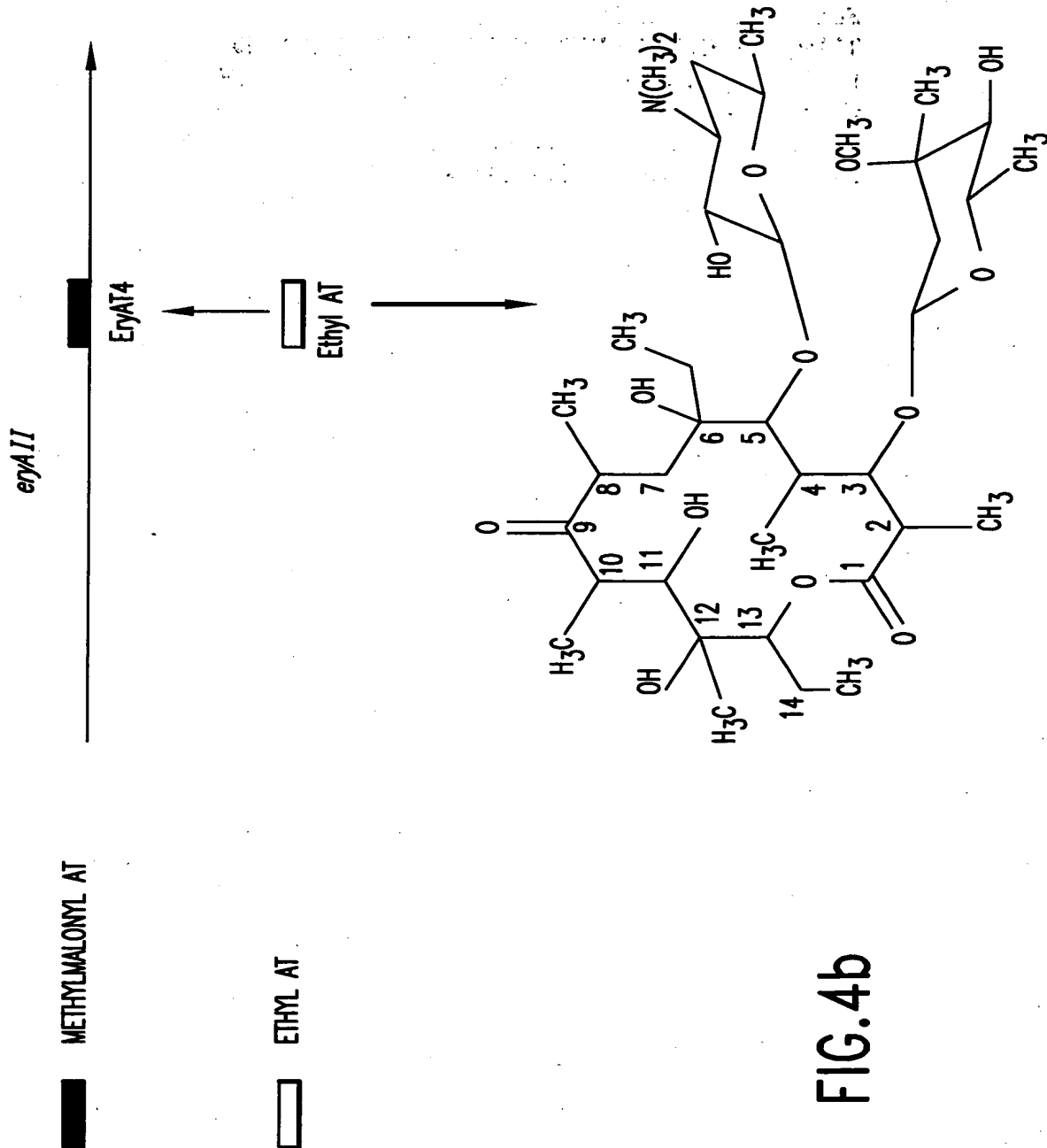


FIG.4b

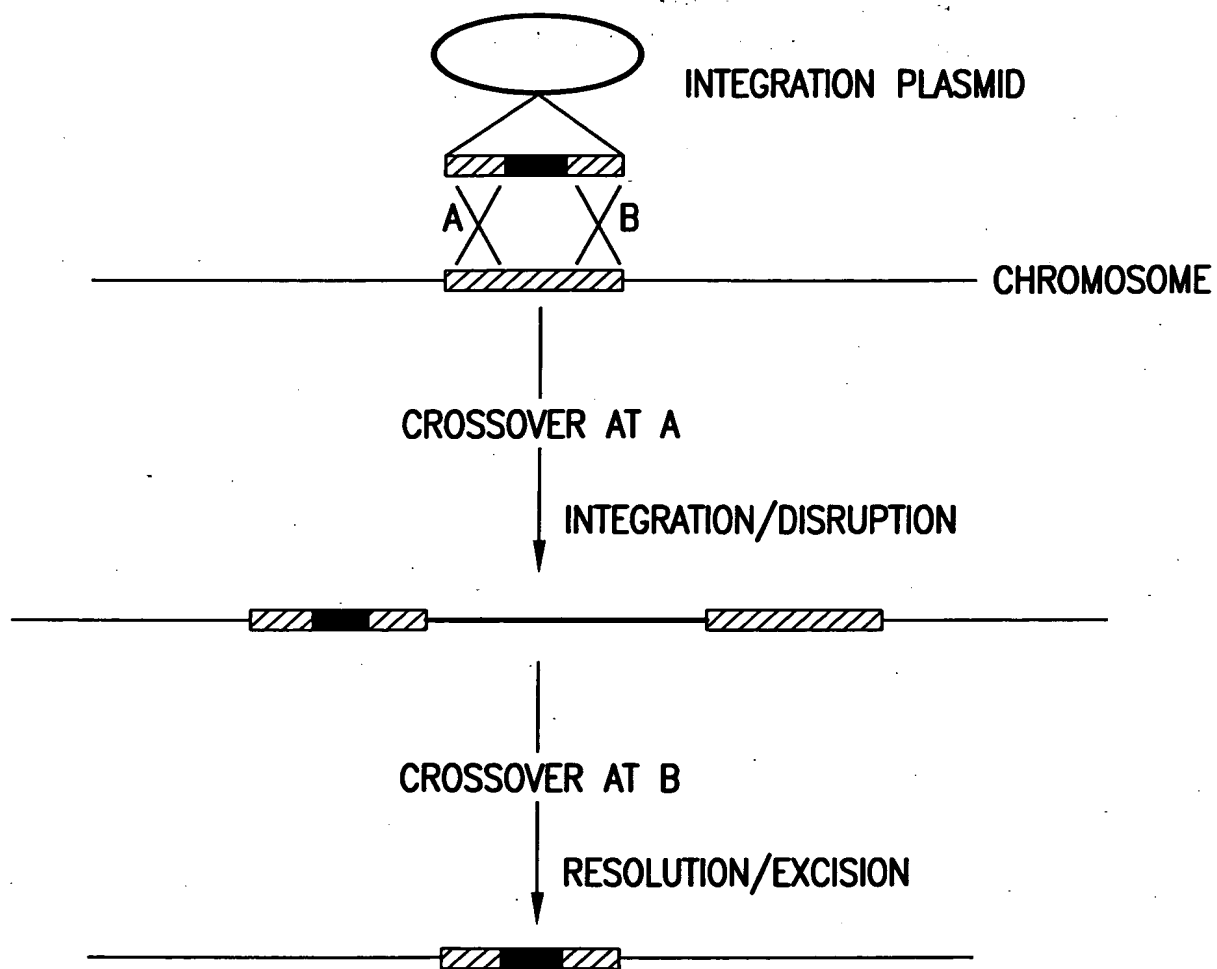


FIG.5



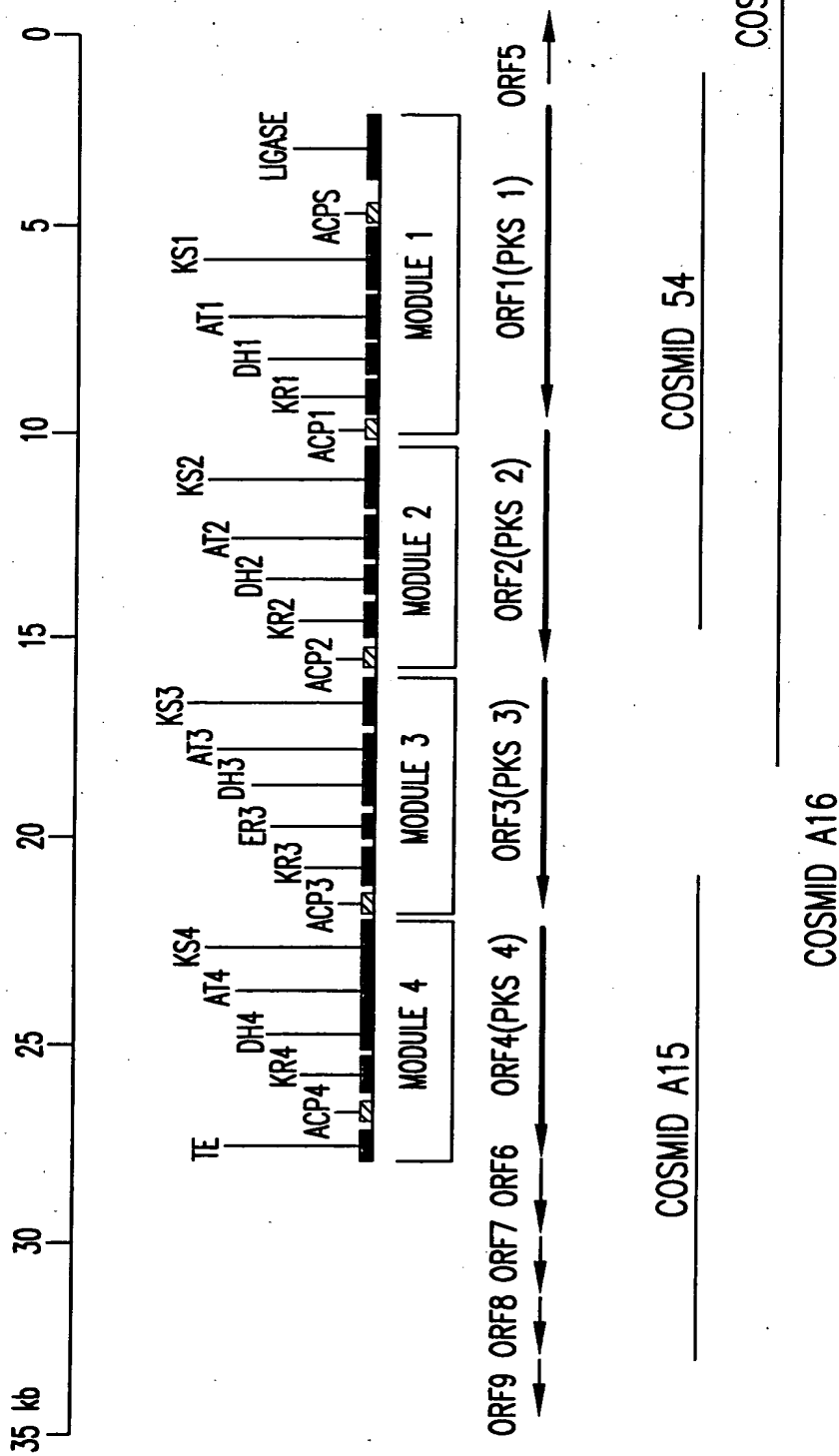


FIG.6

GGGCCGCTGGCGGTGATGTTACCGGACAGGGCTCCCAACGCCCCGGCATGGGACGACAG 60  
 G P L A V M F T G Q G S Q R P G M G R Q 20  
 TTGTACGAGCACTTCCCCGTCTSCGCCCAGGCACTGGACGAGGTCTTCGCACTCGCCACC 120  
 L Y E H F P V F A Q A L D E V F A L A T 40  
 CCCGACTACGCGAGGTGATGTTTCGACCCCGACCAGGGCGAAACACTCCAACGCACCGAC 180  
 P G L R E V M F D P D Q A E T L Q R T D 60  
 CACGCCCAGATCGCCCTGTTCCGCTTCGAAACCGCCCTCTACCGACTCTGGGAATCCTGG 240  
 H A Q I A L F A F E T A L Y R L W E S W 80  
 GGCCTGCGACCCGACATGGTCTGCGGACACTCGGTGCGAGAAATCACCGCAGCCACGTC 300  
 G L R P D M V C G H S V G E I T A A H V 100  
 TCCGGCACCCCTCACCTCCCCGACGCCGTCCACCTCGTCACCACACGCGGCACCCCTCATG 360  
 S G T L T L P D A V H L V T T R G T L M 120  
 CAAAACCTGCCCCCGGGCGGCCATGCTCGCCGTGCCACCGACCCCCACACCCTCCAA 420  
 Q N L P P G G A M L A V A T D P H T L Q 140  
 CCCACCTCGACAACCACGACACCATCTCCATCGCCGCCATCAACGGCCCCCAGGCC 480  
 P H L D N H H D T I S I A A I N G P H A 160  
 ACCGTCTCTCCGGCGACCGCACCCCTCCACCACATCGCCACCCAACCTCAACACCAAA 540  
 T V L S G D R T T L H H I A T Q L N T K 180  
 ACCAACTGGCTCAACGTCAGCCACGCCTTCCACTCCCCCTCATGCAACCCATCCTCCAA 600  
 T N W L N V S H A F H S P L M Q P I L Q 200  
 CCCTTCACCACCACCCTCAACACCCTCACCCACCACCCCCACACACACCCTCATCAGC 660  
 P F T T T L N T L T H H P P H T P L I S 220  
 ATGCTCACCGCCACACCCACCCACCCCGACACCACCCACTGGACCCAGCACATCACCGCA 720  
 M L T A T P T H P D T T H W T Q H I T A 240  
 CCCGTCCGCTACACCGACACCCTCCACCACCTCCACCACCACGGCATCACCACTACCTC 780  
 P V R Y T D T L H H L H H H G I T T Y L 260  
 GAAATCGGCCCCGACACCACCCTCACCGCCCTCGCCCGCACCACCCTCCCCACCACCACC 840  
 E I G P D T T L T A L A R T T L P T T T 280  
 CACCTCATCCCCACCACCCGCCGAACCACAACGAAGTCCGCAGCACGAACGAGGCGTTG 900  
 H L I P T T R R N H N E V R S T N E A L 300  
 GGCAGGGTGTTCAGCGTGGGCCACTCGGTGGACTGGCGGGCCCTCACTCCGACCGGGAGG 960  
 G R V F S V G H S V D W R A L T P T G R 320  
 CGTACCTCCCTGCCGACGTACCCCT 985  
 R T S L P T Y P 328

FIG.7

## PCR OLIGOS:

N-TERMINAL OLIGO: 5' *Eco*RI Tag-<sup>*Avr*II</sup>  
 CCTAG GCTGGCGGTGATGTTCA-3'  
 GGGCC  
 [ENGINEERED *Avr*II] [HOMOLOGOUS REGION]

C-TERMINAL OLIGO: 5' *Bam*HI Tag-<sup>*Nsi*I</sup>  
 ATGCATACGTCGGCAGGGAGGTAC-3'  
 G GG  
 [ENGINEERED *Nsi*I] [HOMOLOGOUS REGION]

## PCR CLONING:

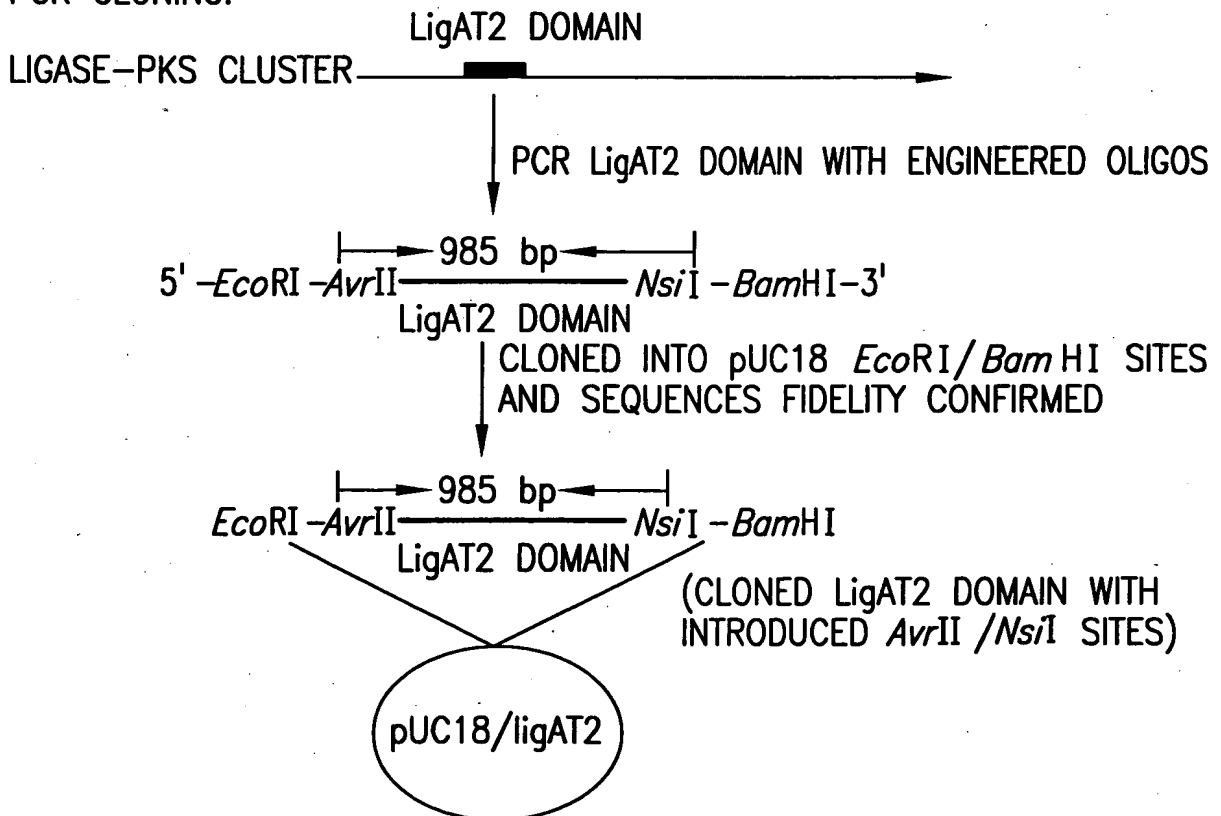


FIG.8

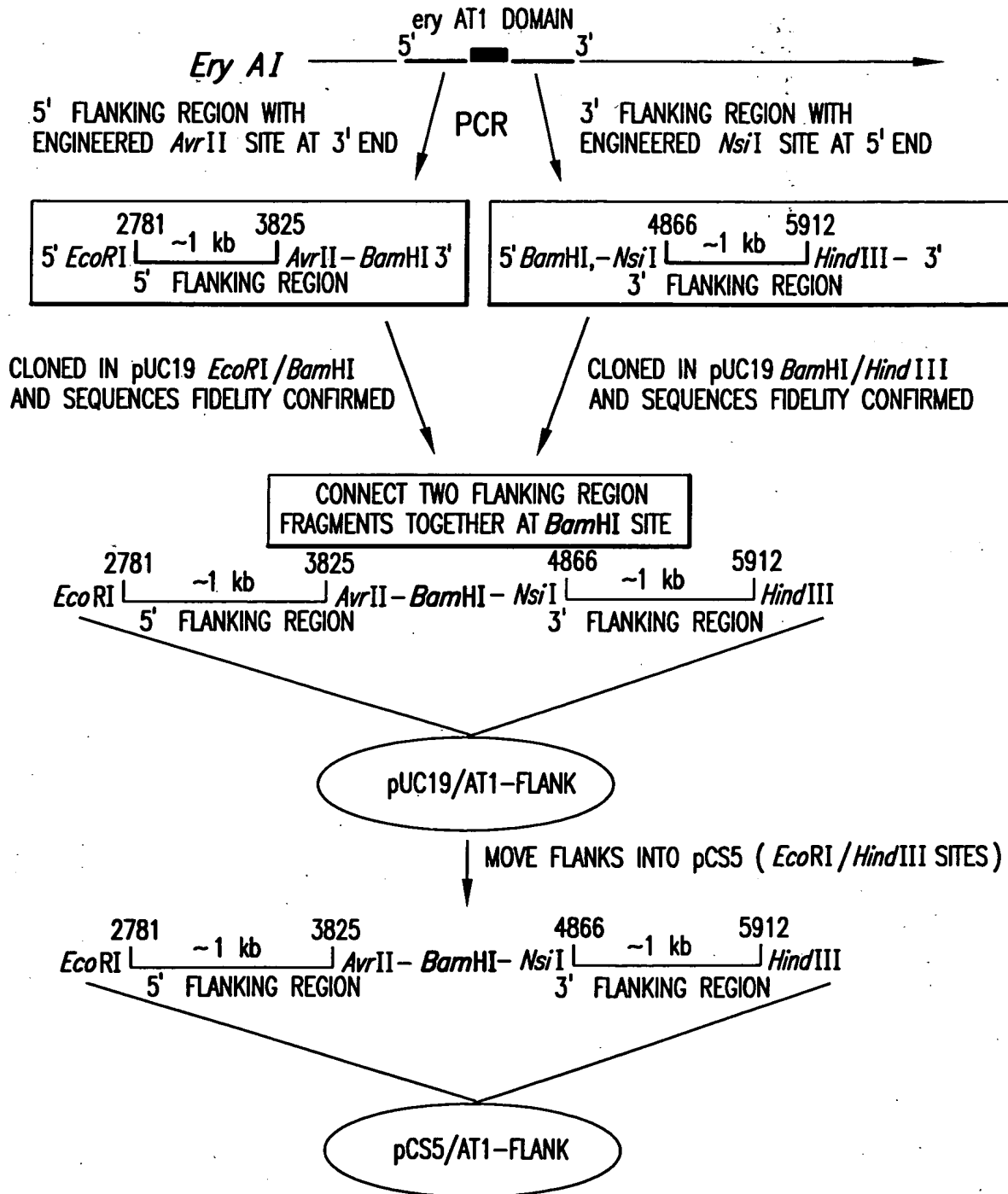


FIG.9

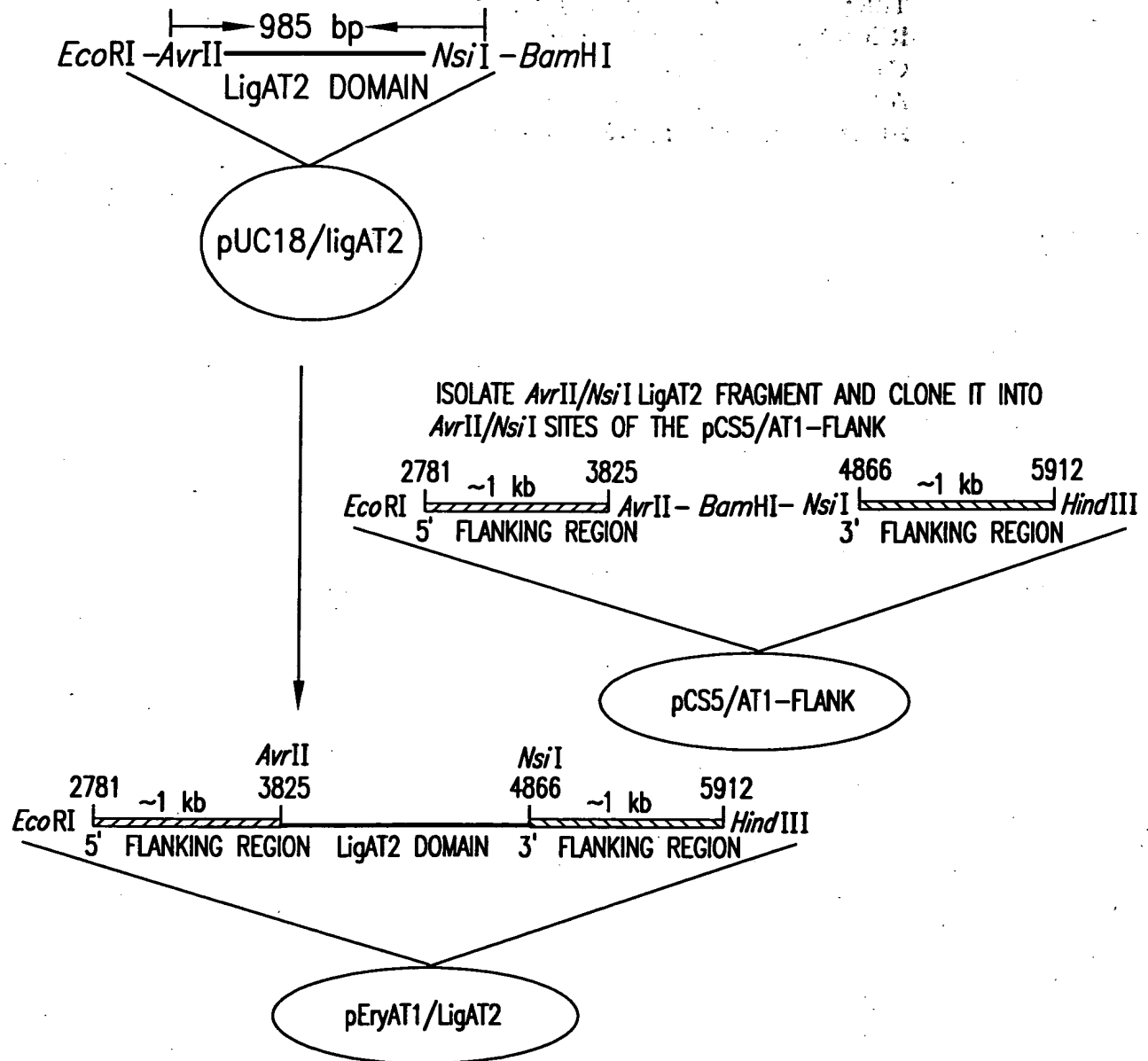


FIG.10

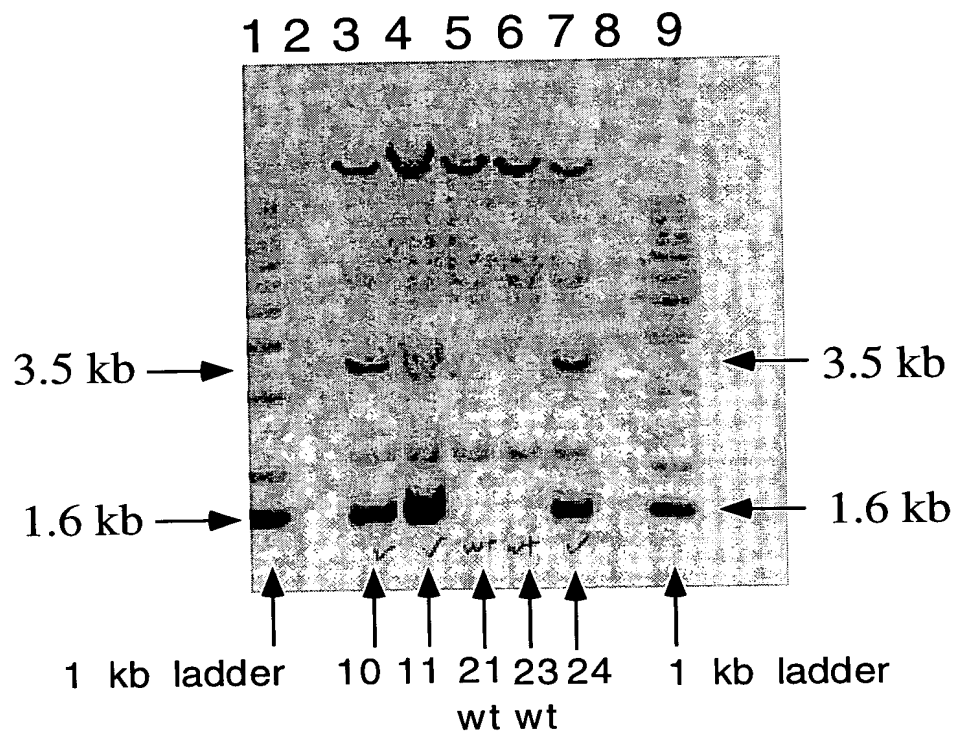


FIG. 11

Do not use the same word more than once in the same paragraph.

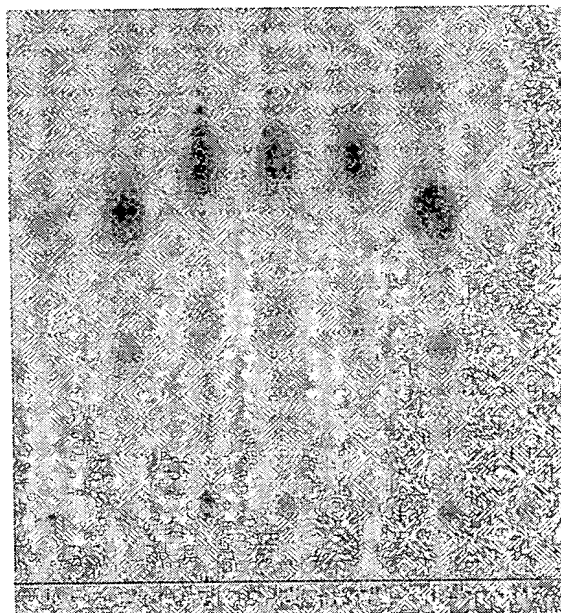


FIG. 12

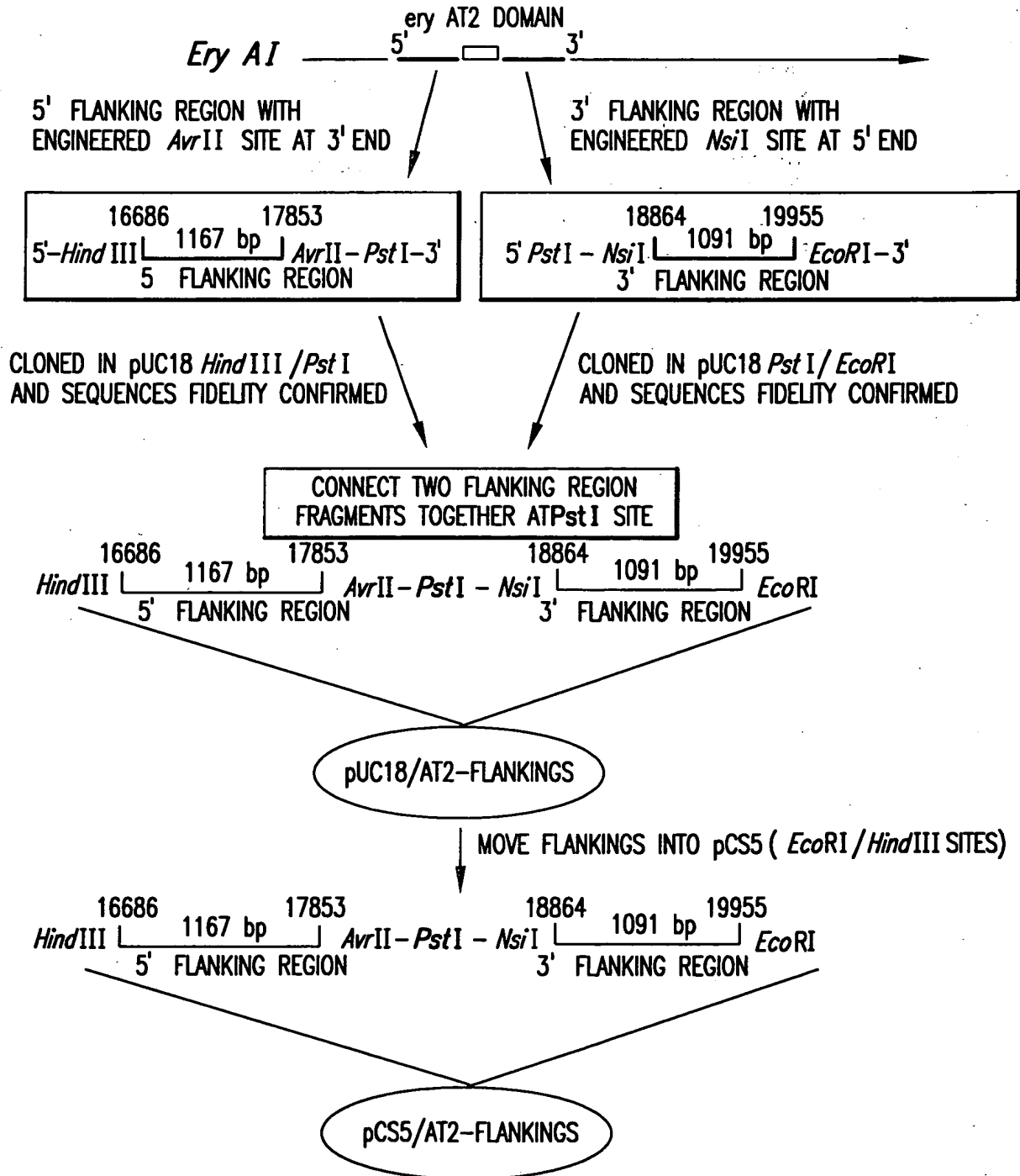
CONSTRUCTION OF *ery* AT2 FLANKING REGIONS IN pCS5

FIG.13



## SCHEME FOR CONSTRUCTION OF pEryAT2/LigAT2 INTEGRATION PLASMID

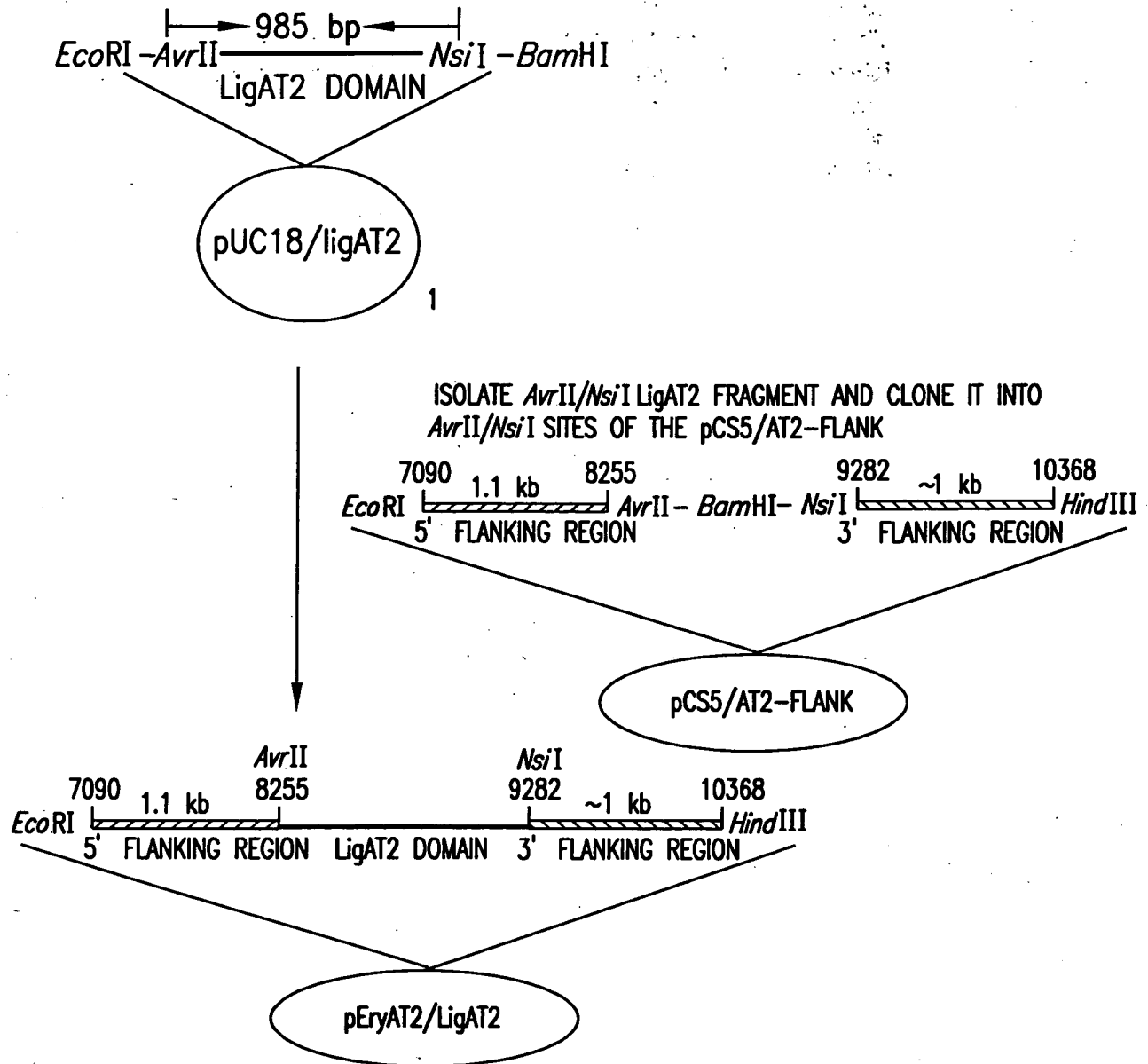


FIG.14

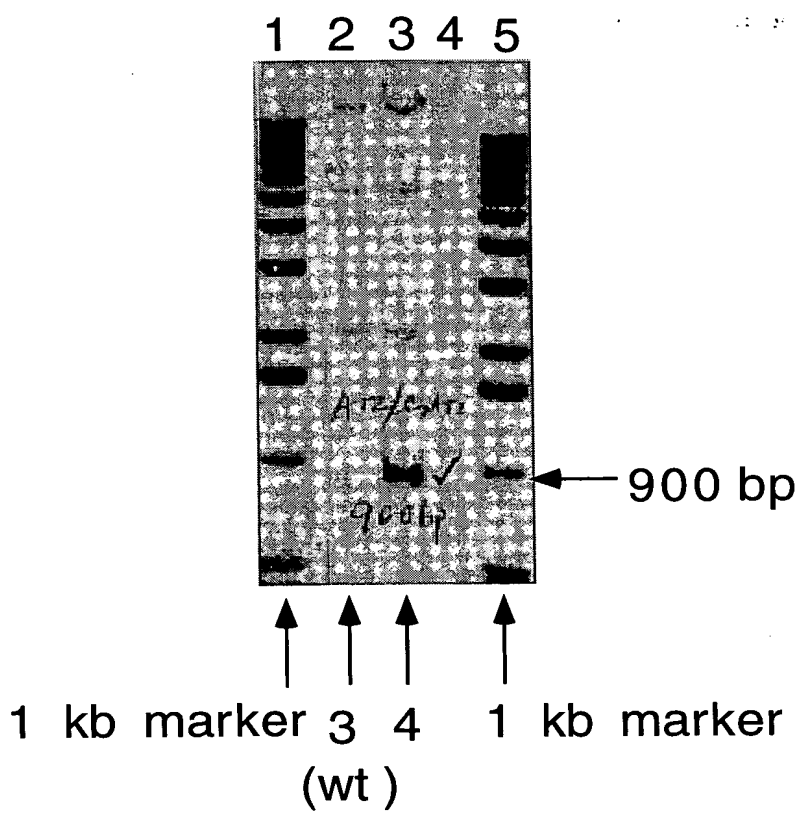


FIG. 15



1 2 3 4 5 6

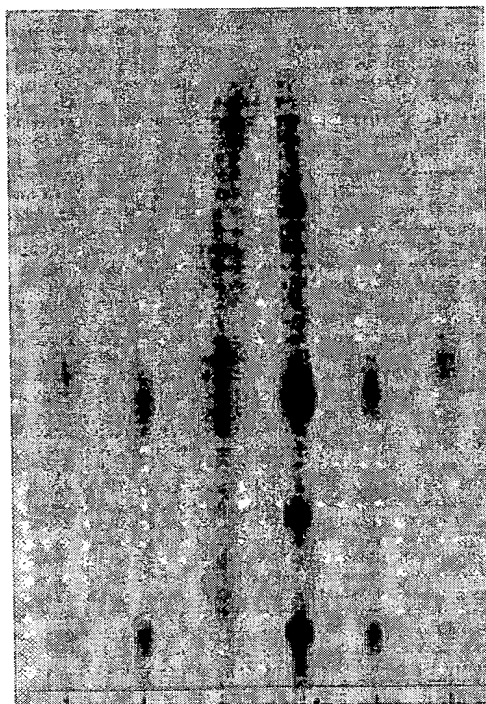


FIG.16

073055, 050341  
"03050" 95055260



95056260

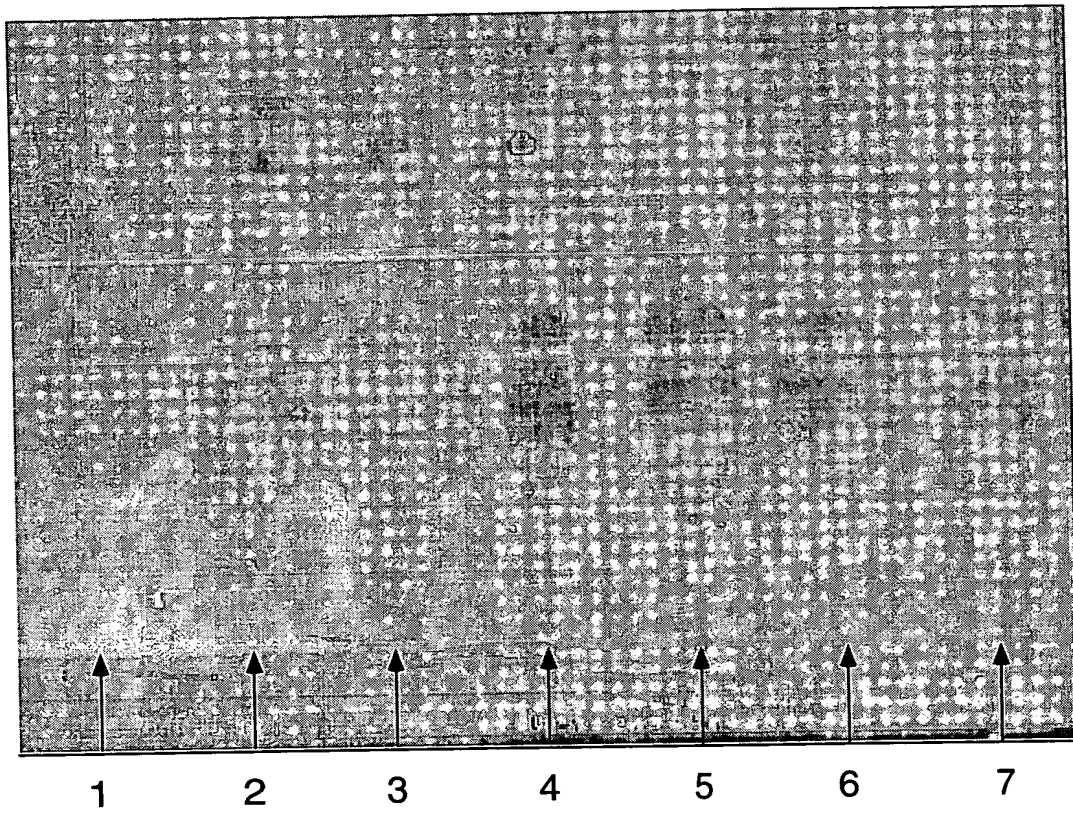


FIG. 17

CCTAGGACGGCAGTCCTGCTCACCAGGGCAGGGTTCCCAGCGTCAGGGCATGGGGCGCGAA 60  
 P R T A V L L T G Q G S Q R Q G M G R E 20  
 CTGTACGACCGGTACCGGTGTTCCGGCCTCGTTGACGCGATCTGCGCTCAACTCGAC 120  
 L Y D R S P V F A A S F D A I C A Q L D 40  
 GGGCAACTGCCTCGTCCCCTCAAGGACGTTCTCTTCGCCCCGAGGGGTCGGAGGACGCC 180  
 G Q L P R P L K D V L F A P E G S E D A 60  
 GCGCTCASCAGCGTACGGTGTTACACAGGCGGCTCTGTTCCCGTGAGACCTCCCTG 240  
 A L I D R T V F T Q A A L F A V E T S L 80  
 TTCCGGCTGTTGAGGCCCACGGCCTCGSCCCGACTACCTCASCGGCCACTCCATCGGC 300  
 F R L F E A H G L V P D Y L I G H S I G 100  
 GAAGTGACCGCGGCCCGCTGCCCGGGTCTCGATCTGGCGGACGCGTCCGTCTGGTC 360  
 E V T A A H L A G V L D L A D A C V L V 120  
 GCCCACC CGCGCCGCTGATGCAGTCGGCCCGGGCCGGCGCGGATGGCCCGGTCCAG 420  
 A H R G R L M Q S A R A G G A M A A V Q 140  
 GCGAGCGAGGACGAGGTACGCGAGGCCCTCGCGACCTTCGACGATGCGGTTCCCGTGCC 480  
 A S E D E V R E A L A T F D D A V A V A 160  
 GGAGTCAACGGCCCCGAACGCCACCGTCGTCTCCGGCGACGAGGACGCGGTGAGCGGCTG 540  
 G V N G P N A T V V S G D E D A V E R L 180  
 GTCCGCGCTGGCGGAGCAGGGCAGGCGGACGAAGCGGCTGCCGGTCAGCCACGCCTTC 600  
 V A R W R E Q G R R T K R L P V S H A F 200  
 CACTCGCCGCACATGGACGGGATCGTCGACGAGTTCTGTCACCGCCGTCTCCGGGCTCACC 660  
 H S P H M I G I V D E F V T A V S G L T 220  
 TTCCGCTCCCCGACGLTCCCGGTGCTCTCCAACGTACCGGGACCCTCGCCACCGTCGAC 720  
 F R S P T I P V V S N V T G T L A T V D 240  
 CACCTGACCTCGCCCGCTACTGGGCACGCCACATCCGGAGGCCGTGCGCTTCGCCGAC 780  
 Q L T S P A Y W A R H I R E A V R F A D 260  
 GGGGTGCGGTACCTGGAGGGCGAGGGCGTCACCGAATGGCTGGAGCTCGGGCCCCGACGGC 840  
 G V R Y L E G E G V T E W L E L G P D G 230  
 GTTCTCGTCGCCCTGGTCGAGGACTGCCCTGGCGAAGGAGGCGGATCGCTCGCGTCCGCC 900  
 V L V A L V E D C L A K E A G S L A S A 300  
 CTGCGCAAGGGGGCGAGCGAGCCCCACACCGTGGGCGCGGCCATGGCCCGCGCGGTGCTG 960  
 L R K G A S E P H T V G A A M A R A V L 320  
 CGCGATCCGGCCCCGACTGGGCGGCGGTGTTCCCGGGCGACGCGGGTTCGACCTCCG 1020  
 R G S G P D W A A V F P G A R R V D L P 340  
 ACGTATGCAT 1030  
 T Y A 343

FIG. 18

N-TERMINAL OLIGO: 5' *Eco*RI Tag-CCTA <sup>*Avr*II</sup>GGACGGCAGTCCTGCTCACC-3'  
GGCC  
 | ENGINEERED *Avr*II | | HOMOLOGOUS REGION |

C-TERMINAL OLIGO: 5' BamHI Tag— $\overbrace{\text{ATGCATACGTCGGAAGGTCGACCCG}}^{\text{NsiI}}$ —3'  
                                C       C  
                    | ENGINEERED NsiI || HOMOLOGOUS REGION |

OR CLONING:

Ven-PKS CLUSTER

venAT DOMAIN

PCR venAT DOMAIN WITH ENGINEERED OLIGOS

5' -EcoRI Tag- AvrII 1030 bp NsiI -BamHI Tag-3'

venAT DOMAIN

CLONED INTO pUC18 *HincII* SITES AND SEQUENCES FIDELITY CONFIRMED

[*HincII*] EcoRI -AvrII 1030 bp NsiI -BamHI [*HincII*]

venAT DOMAIN

(CLONED venAT DOMAIN WITH INTRODUCED *AvrII* /*NsiI* tag)

pUC18/venAT

FIG. 19

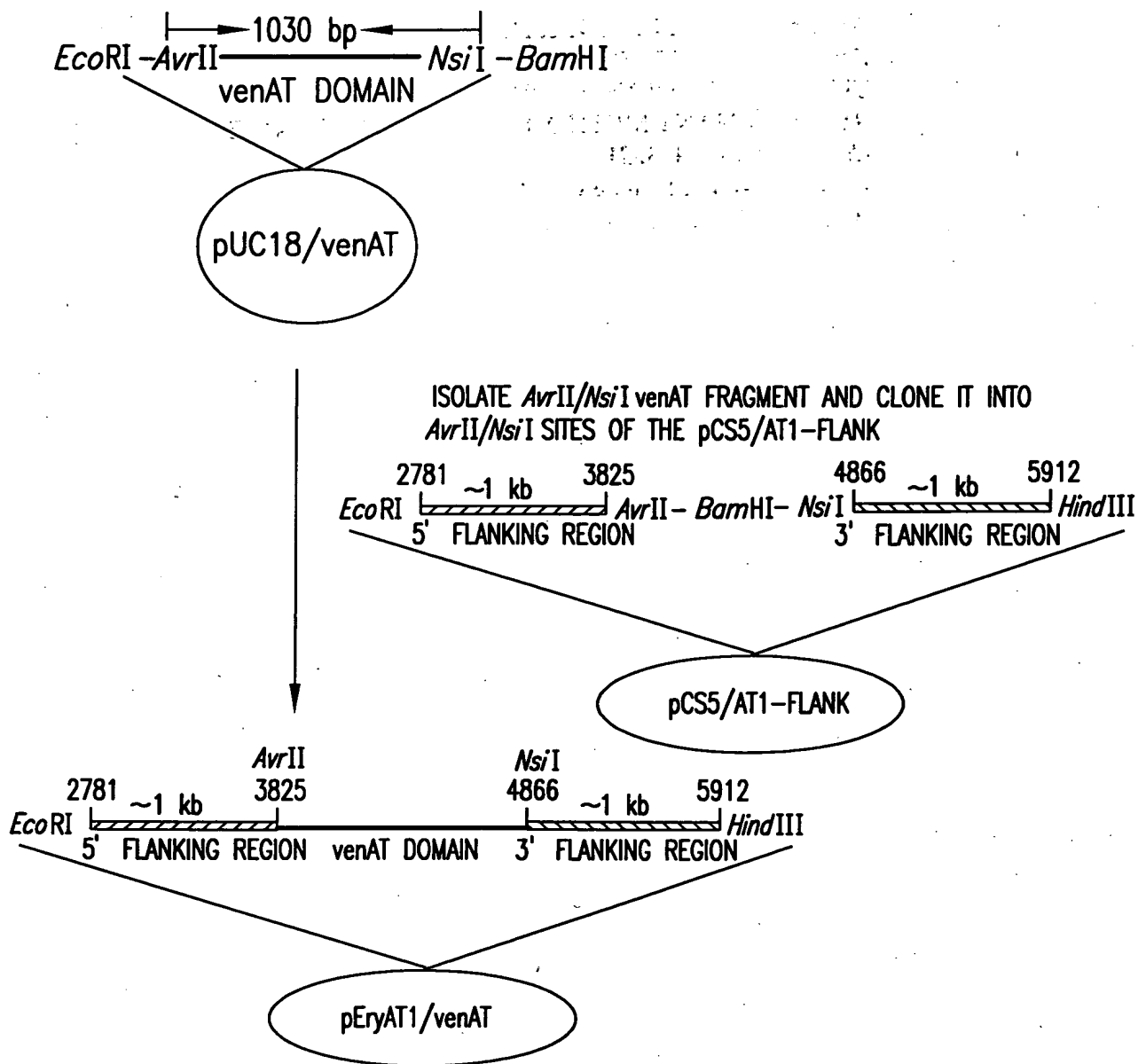


FIG.20

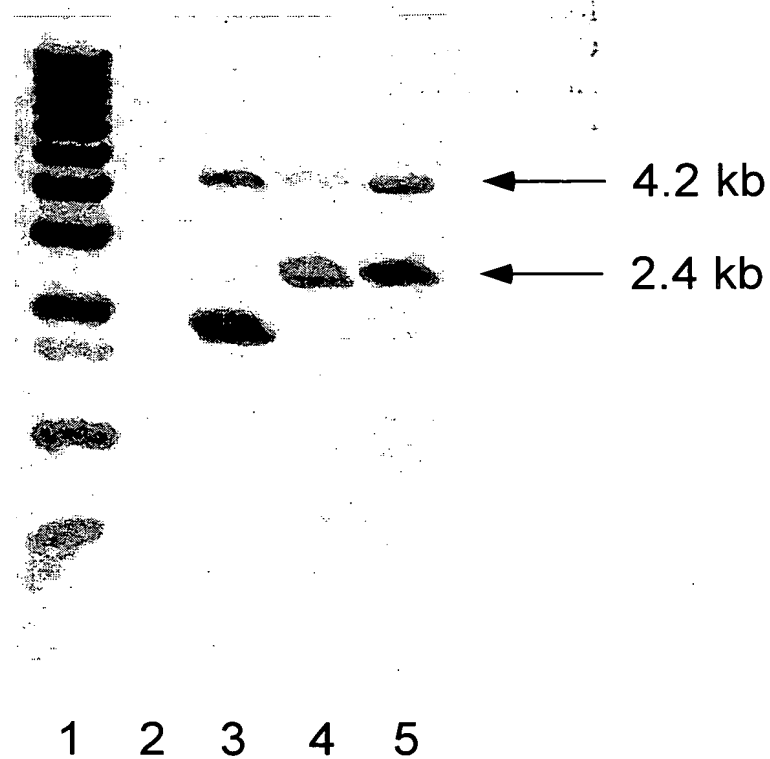


FIG. 21



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MEB

EryA



1 2

FIG. 22

## PCR OLIGOS:

N-TERMINAL OLIGO: 5' *Eco*RI Tag-<sup>*Avr*II</sup>  
<sup>GGC C</sup>  
 CCTAGGGTTGCCTTCCTGTTTCGAC-3' (SEQ. ID NO. 17)

ENGINEERED *Avr*II | HOMOLOGOUS REGION

C-TERMINAL OLIGO: 5' *Hind*III Tag-<sup>*Nsi*I</sup>  
<sup>C G</sup>  
 ATGCATAGACCGGCAGATCCACCG-3' (SEQ. ID NO. 18)

ENGINEERED *Nsi*I | HOMOLOGOUS REGION

## PCR CLONING:

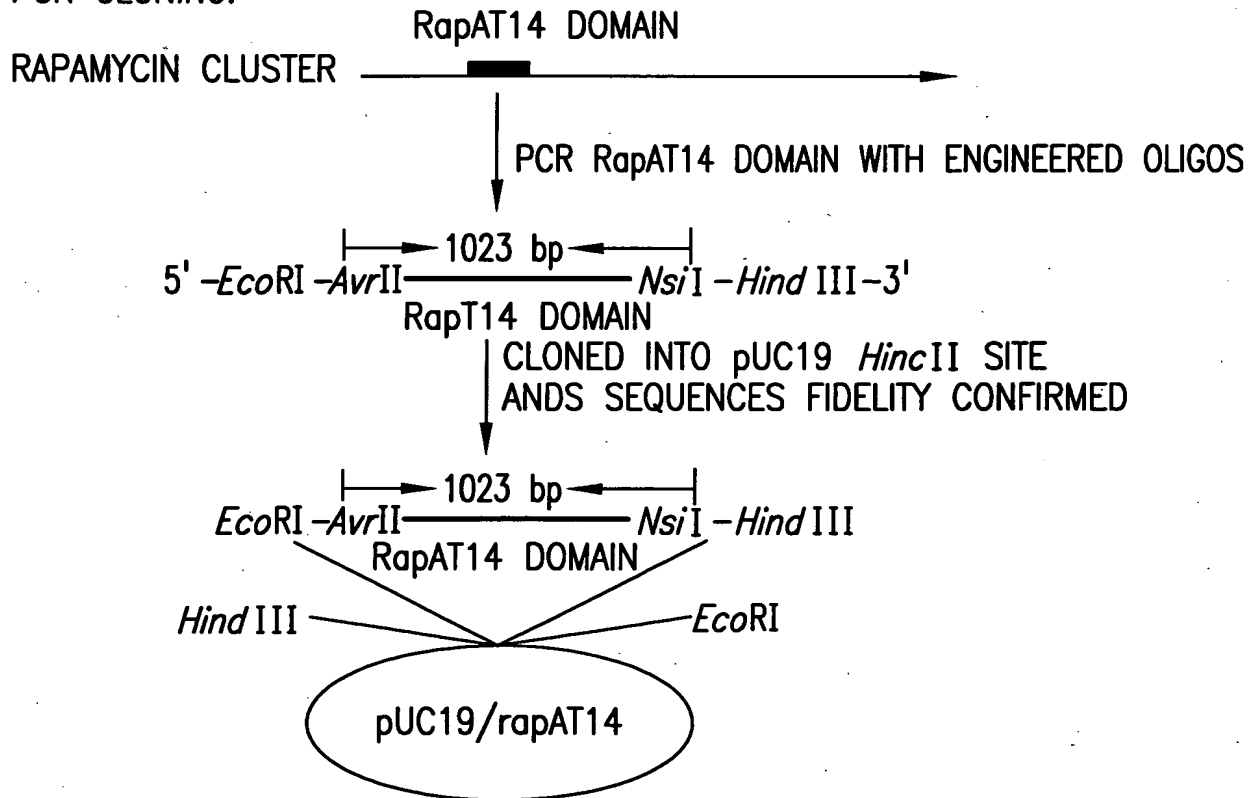


FIG.23

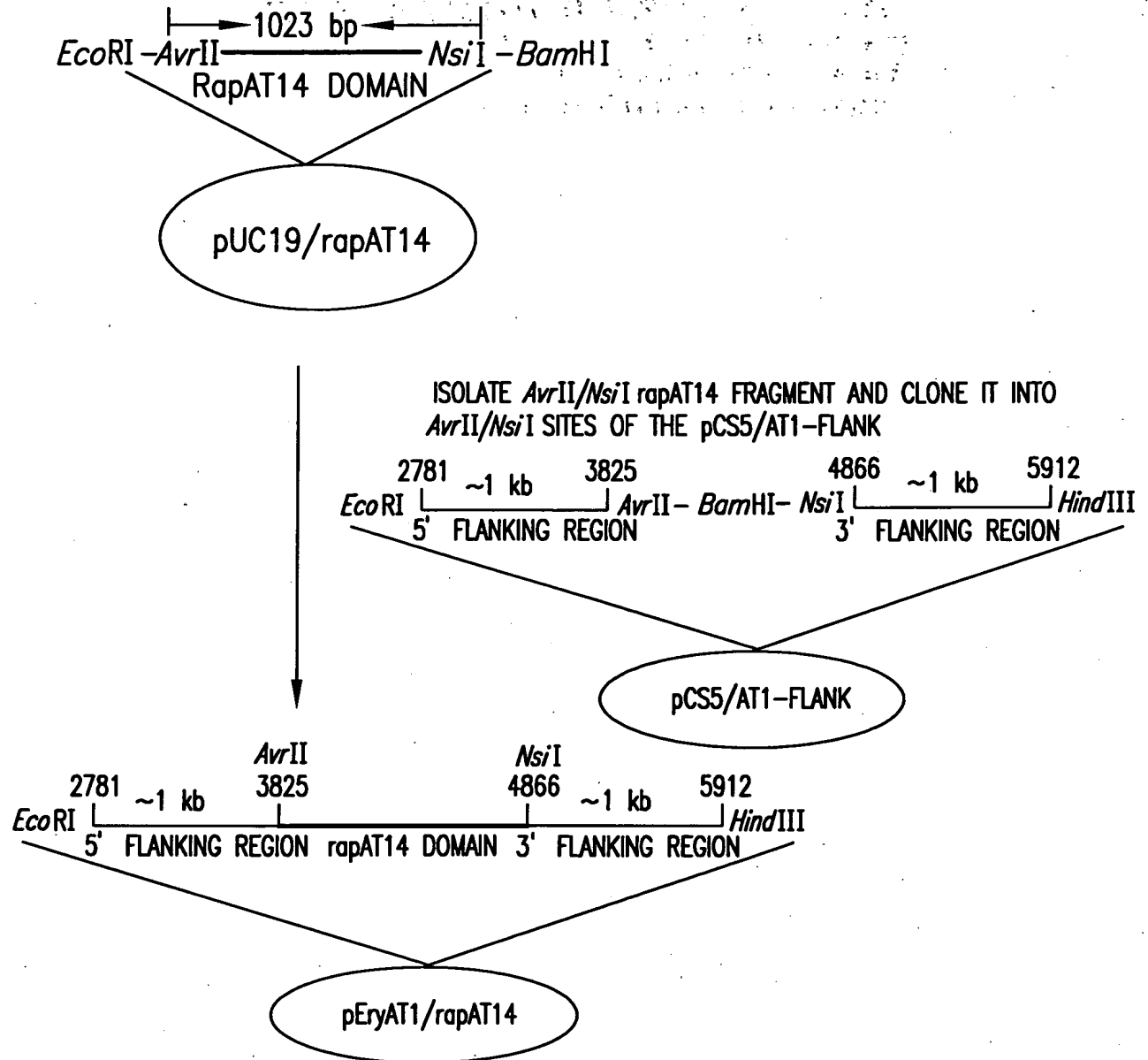


FIG.24

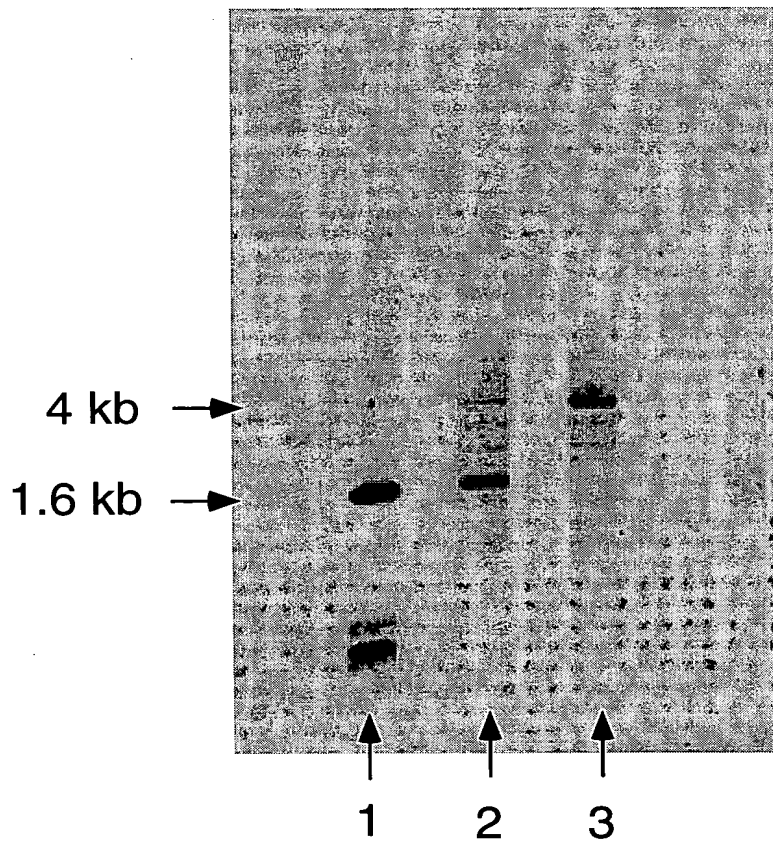


FIG. 25

MEMPHIS, TENN. 38001  
APR 4 1968  
OFFICE



FIG. 26

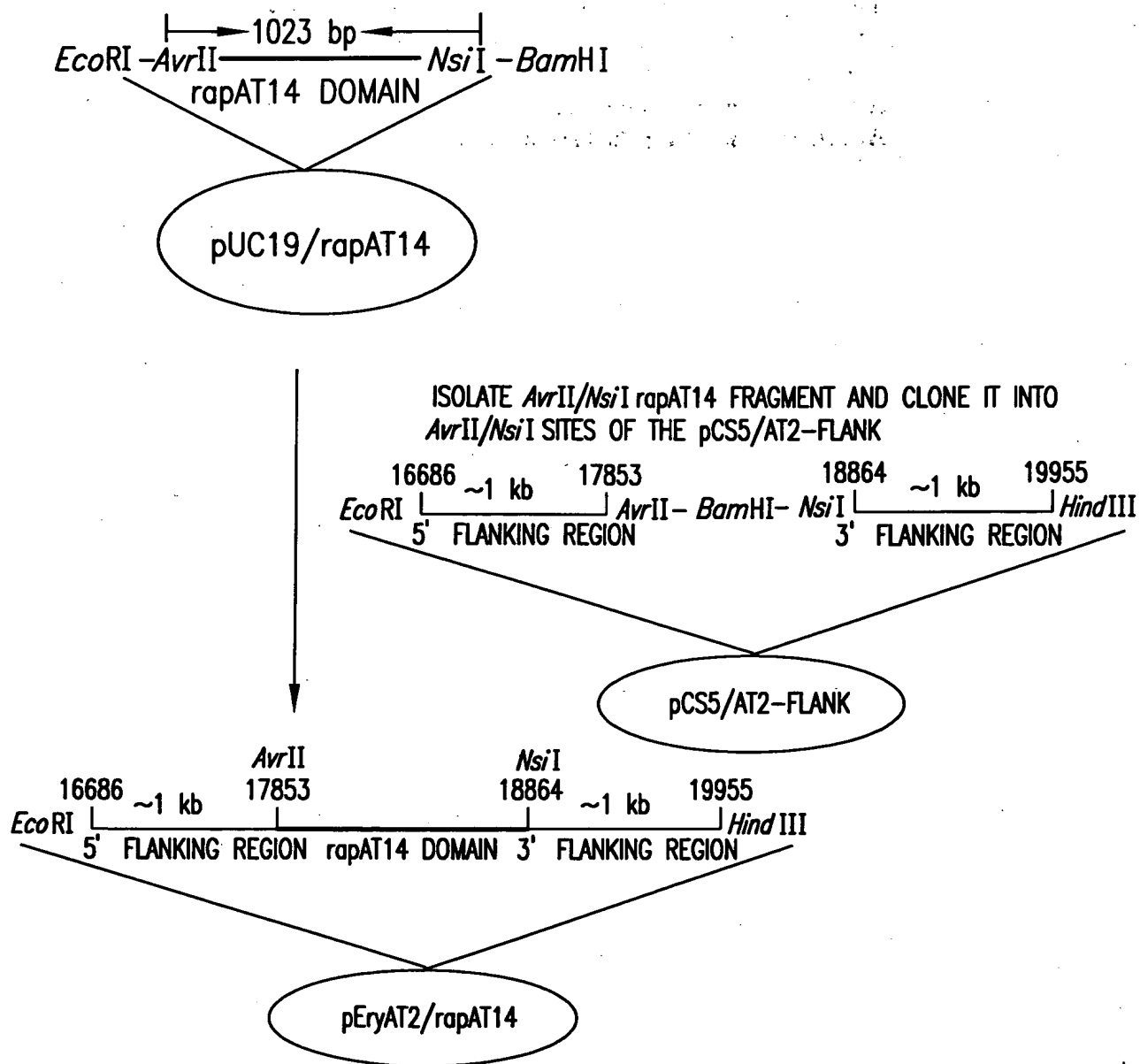


FIG.27

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← 4.3 kb

← 1.1 kb

1 2 3 4 5 6 7

FIG. 28

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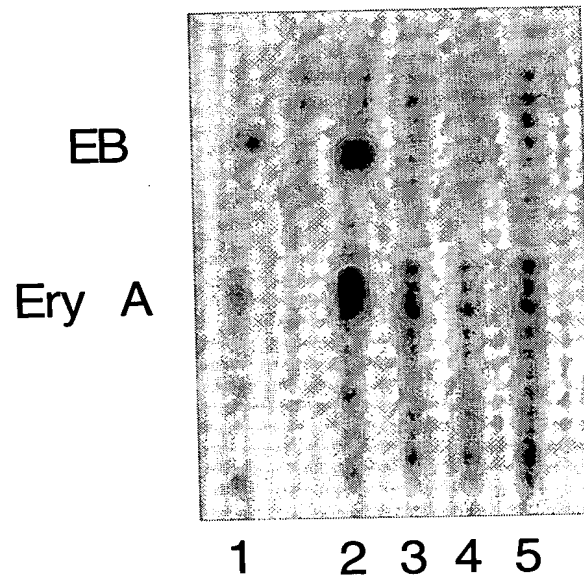


FIG. 29





960950 960950 960950

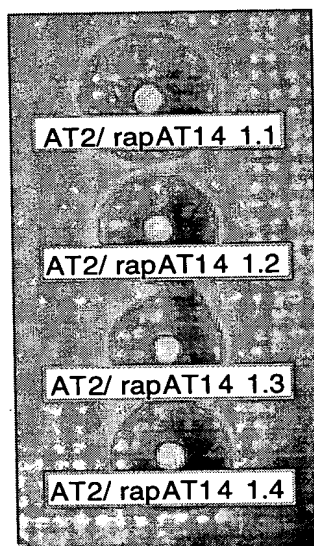


FIG. 30

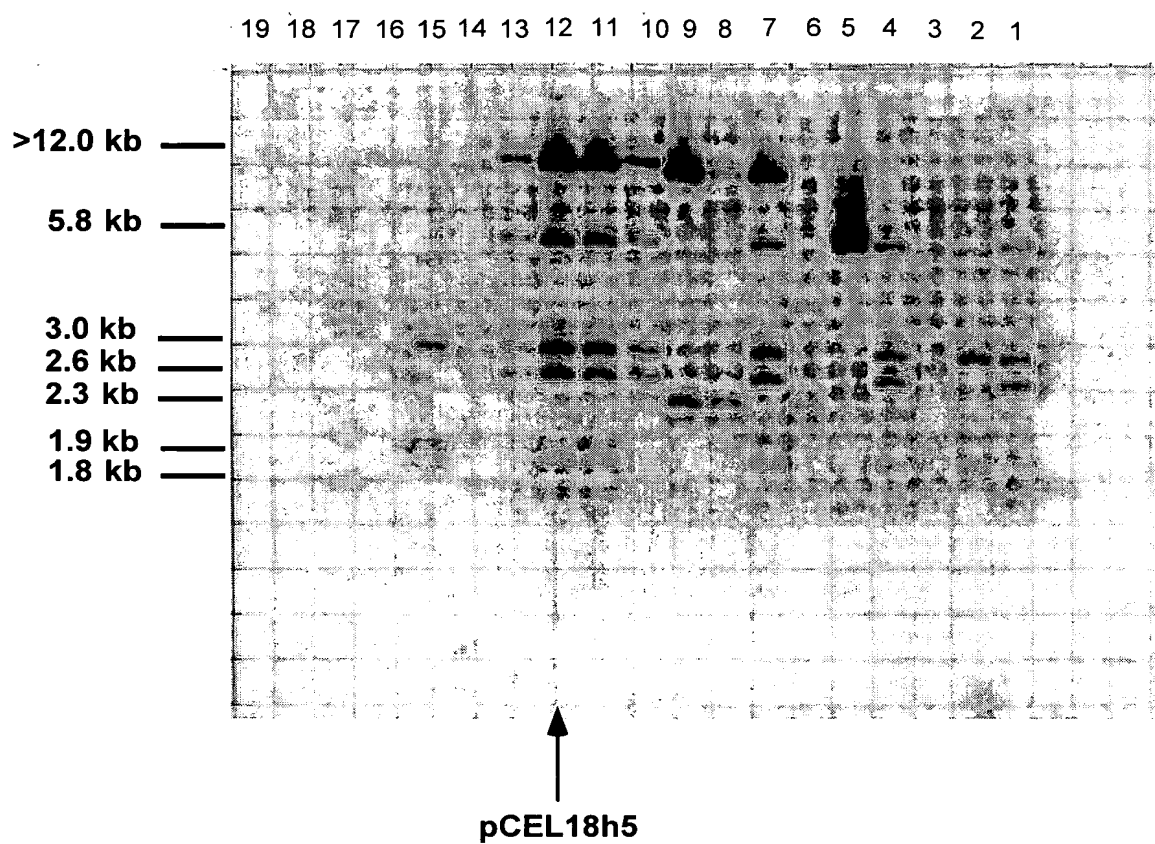
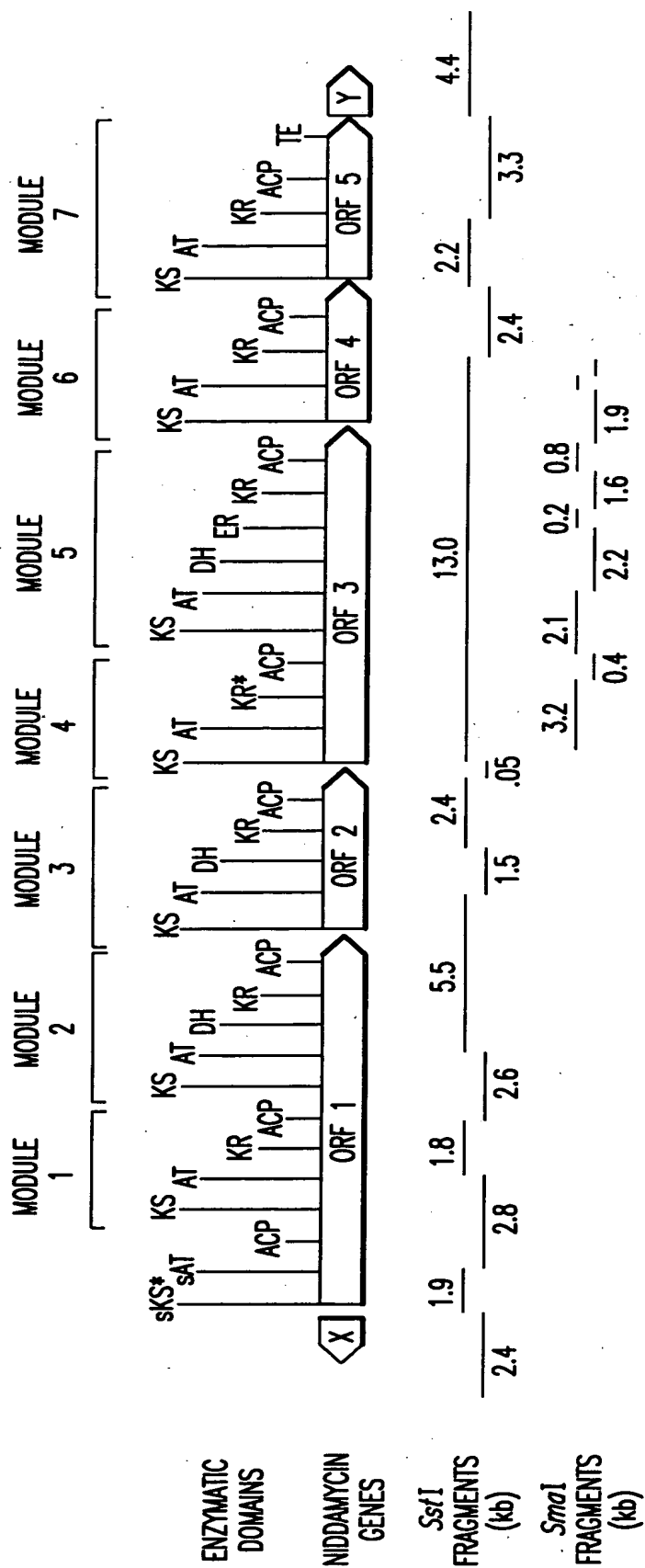


FIG. 31



pCEL18h5

pCEL13f5

FIG.32

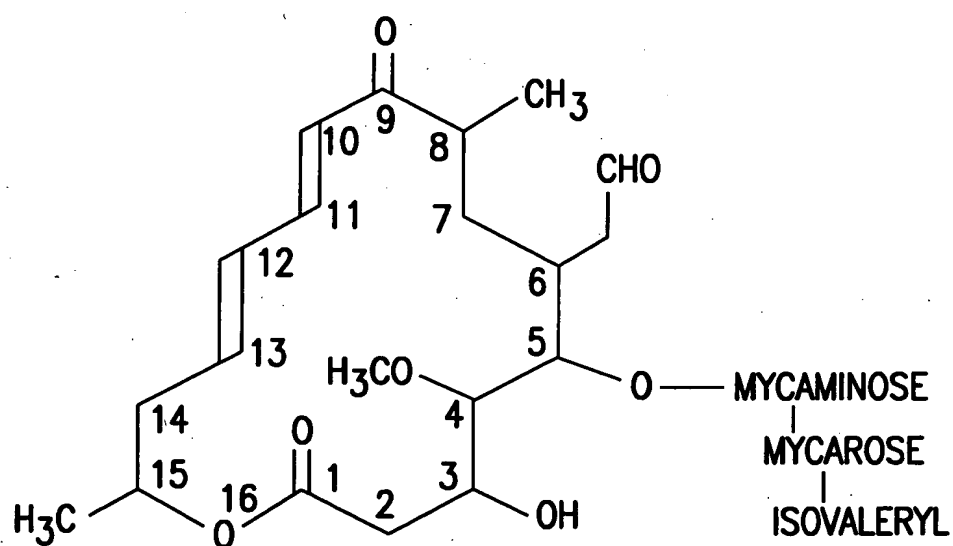
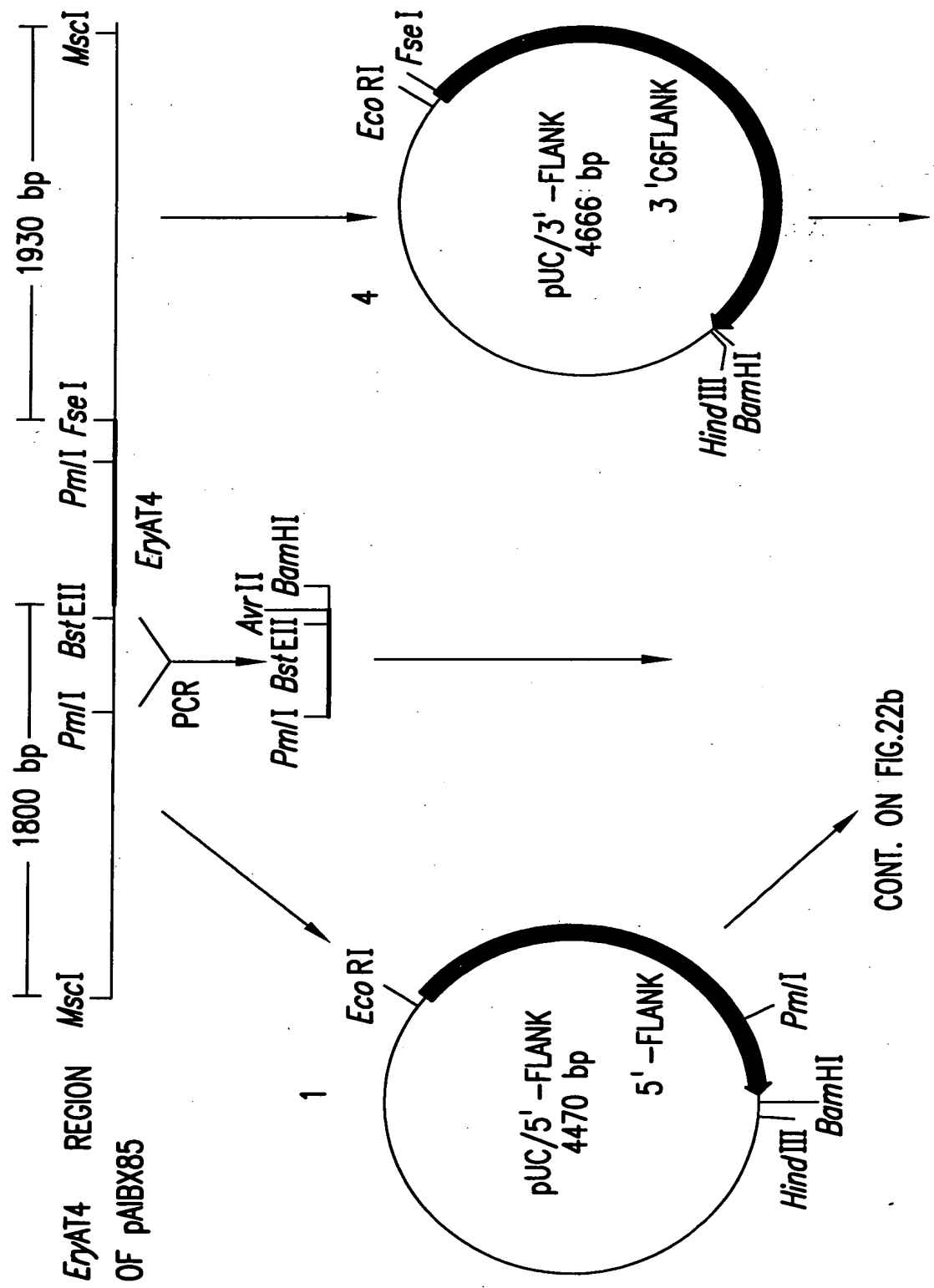


FIG.33

GCCGACCGTGTCGTGTTTCGTGTTCCCCGGCCAGGGCTCGCAGTGGGCCGGAATGGCCGAG 60  
 A D R V V F V F P G Q G S Q W A G M A E 20  
 GGGCTGCTGGAGCGGTCCGGCGCGTTCGGAGTGGGCCGACTCGTGCGACGCCGCGCTG 120  
 G L L E R S G A F R S A A D S C D A A L 40  
 CGGCCGTACCTCGGCTGCTCGGTGCTGAGCGTGTGCGCGGGGAACCGGACGCGCCCTCG 180  
 R P Y L G W S V L S V L R G E P D A P S 60  
 CTCGACCGGTCGACGTGCTGCAGCCGGTGTGTTACGATGATGGTCTCGCTCGCGGCG 240  
 L D R V D V V Q P V L F T M M V S L A A 80  
 GTCTGGCGTGGCTGGGCGTGGAACCGGCGCGGTGCTCGGGCACTCGCAGGGTGAGATC 300  
 V W R A L G V E P A A V V G H S Q G E I 100  
 GCCGCTGCCCATGTGCGCGGTGCGCTGTGCTGGACGACTCGGCCCCGATCGTCCGCTG 360  
 A A A H V A G A L S L D D S A R I V A L 120  
 CGCAGTCGGGCGTGGCTCGGACTGGCGGGCAAGGGCGGCATGGTGGCGGTGCCGATGCCG 420  
 R S R A W L G L A G K G G M V A V P M P 140  
 GCGGAGGAGCTGCGGCCGCGGCTGGTGACGTGGGGGGACCGTCTGGCCGTGCGCGCCGTC 480  
 A E E L R P R L V T W G D R L A V A A V 160  
 AACAGCCCCGGTTCCTGCGCCGTGCGAGGCGACCCGGAGGCGCTGGCCGAACCTGGTGGCG 540  
 N S P G S C A V A G D P E A L A E L V A 180  
 CTGCTGACCGGTGAGGGGTGCACGCCCCGGCGATCCCCGGCGTGCACACGGCGGGCCAC 600  
 L L T G E G V H A R P I P G V D T A G H 200  
 TCGCCGCAGGTGGACGCGTTGCGGGCTCATCTGCTGGAGGTGCTGGCCCCGGTCCGCCCC 660  
 S P Q V D A L R A H L L E V L A P V A P 220  
 CGACCGGCCGACATCCCGTTCTACTCGACGGTGACCGGCGGGCTGCTGGACGCCACCGAG 720  
 R P A D T P F Y S T V T G G L L D G T E 240  
 CTGGACGCGACGTACTGGTACCGCAACATGCGCGAGCCCGTCGAGTTCGAGCGGCCACA 780  
 L D A T Y W Y R N M R E P V E F E R A T 260  
 CGGGCGCTGATCGCCGACGGGCACGACGTCTTCCTGGAGACGAGCCCGCATCCCATGCTG 840  
 R A L I A D G H D V F L E T S P H P M L 280  
 GCCGTGGCGCTGGAGCAGACGGTCACCGACGCGCGCACCGACGCGGCGGTGCTCGGGACC 900  
 A V A L E Q T V T D A G T D A A V L G T 300  
 CTGCGCCGCGCCACGGCGGTCTCGCGCGCTGGCCCTGGCCGTCTGCCGCGCCTTCGCG 960  
 L R R R H G G P R A L A L A V C R A F A 320  
 CACGGCGTGGAGGTGGACCCCGAGGCGGTCTTCGGTCCGGGCGCACGGCCCGTGGAGTTG 1020  
 H G V E V D P E A V F G P G A R P V E L 340  
 CCCACCTATCCG 1032  
 P T Y P 344

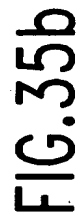
FIG. 34



CONT. ON FIG.22b

CONT. ON FIG.22b

FIG.35a



PROTEIN SEQUENCE	S   A   P   R   K   P
ORIGINAL SEQUENCE	TCCGCGCCGCGCAAGCCG
	↓ ↓ ↓
ALTERED SEQUENCE	TCCGCGCCTAGGAAGCCG
	└──────────┘
	AvrII SITE

PCR OLIGOS FOR 5'-FLANK *AvrII* SITE

N-TERMINAL OLIGO 5'-GAGAGAGGAACCAACGCGCACGTGATCGTCAAGAGGCACCAGC (SEQ. ID. NO. 21)	┌──────────┐ └──────────┘ <i>PmII</i> SITE	5'- FLANK SEQUENCE
C-TERMINAL OLIGO 5'-GAGAGAGGATCCGACCTAGGCGCGGAGGTCACCGGCGCGACGGCG (SEQ. ID. NO. 22)	┌──────────┘ └──────────┘ <i>Bam</i> HI SITE <i>AvrII</i> SITE	5'-FLANK SEQUENCE

PCR OLIGOS FOR *NidAT5* FRAGMENT

N-TERMINAL OLIGO 5'-GAGAGACCTAGGAAGCCGGTGTTCTGTTCCCCGGCCAGGGCT (SEQ. ID. NO. 23)	┌──────────┐ └──────────┘ <i>AvrII</i> SITE	BEGINNING OF <i>NidAT5</i>
C-TERMINAL OLIGO 5'-GAGAGAGGATCCGAGGCCGCGCCGTGCGCCCGGACCGAAGACCGCCTC (SEQ. ID. NO. 24)	┌──────────┘ └──────────┘ <i>Bam</i> HI SITE <i>FseI</i> SITE	3' END OF <i>NidAT5</i>

FIG.36



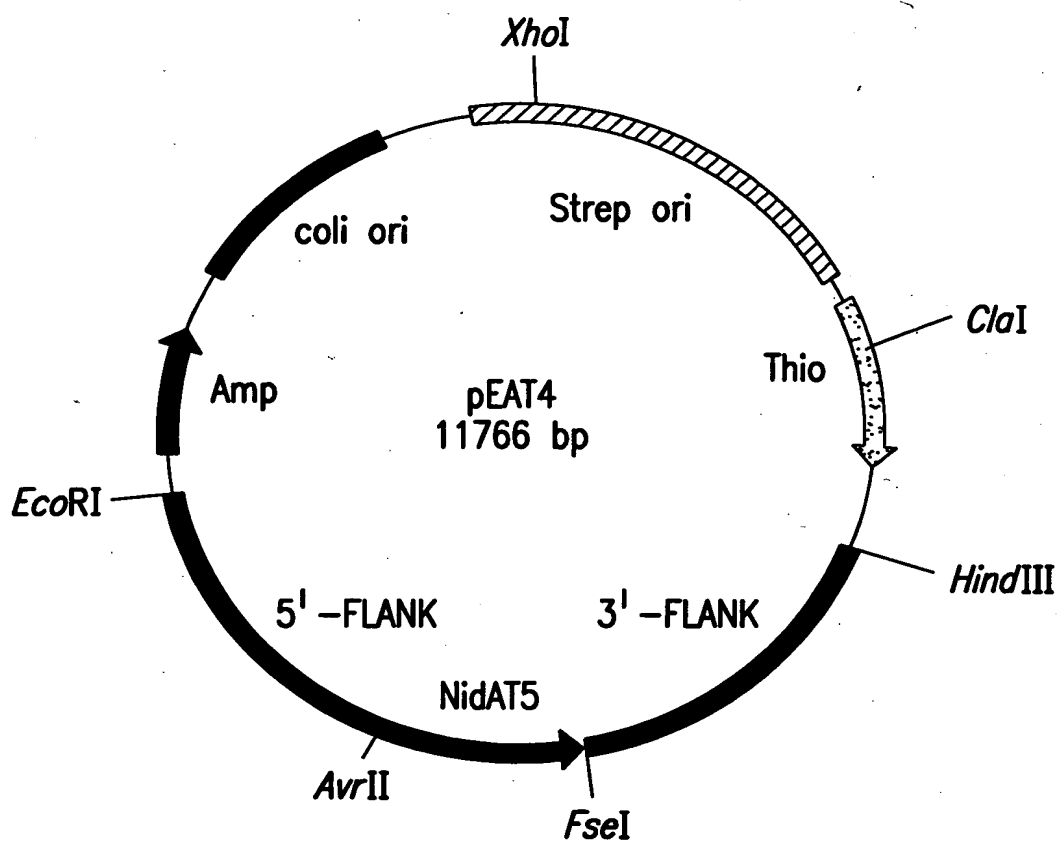


FIG.37

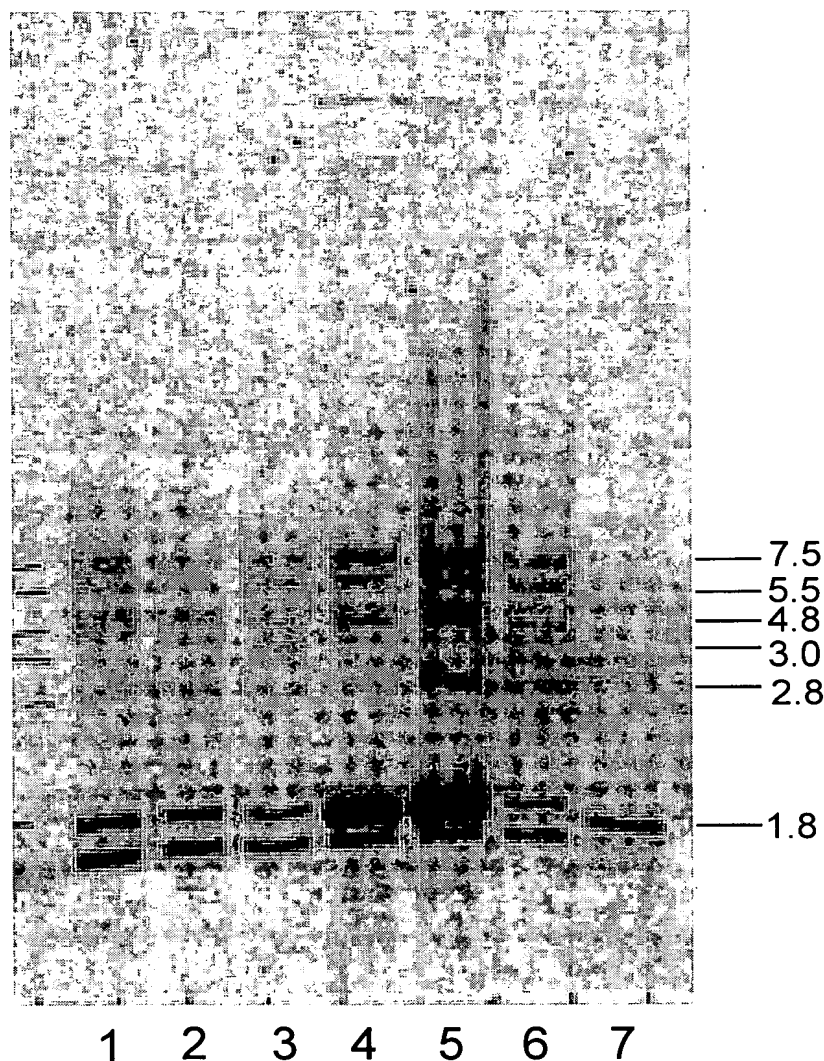


FIG. 38

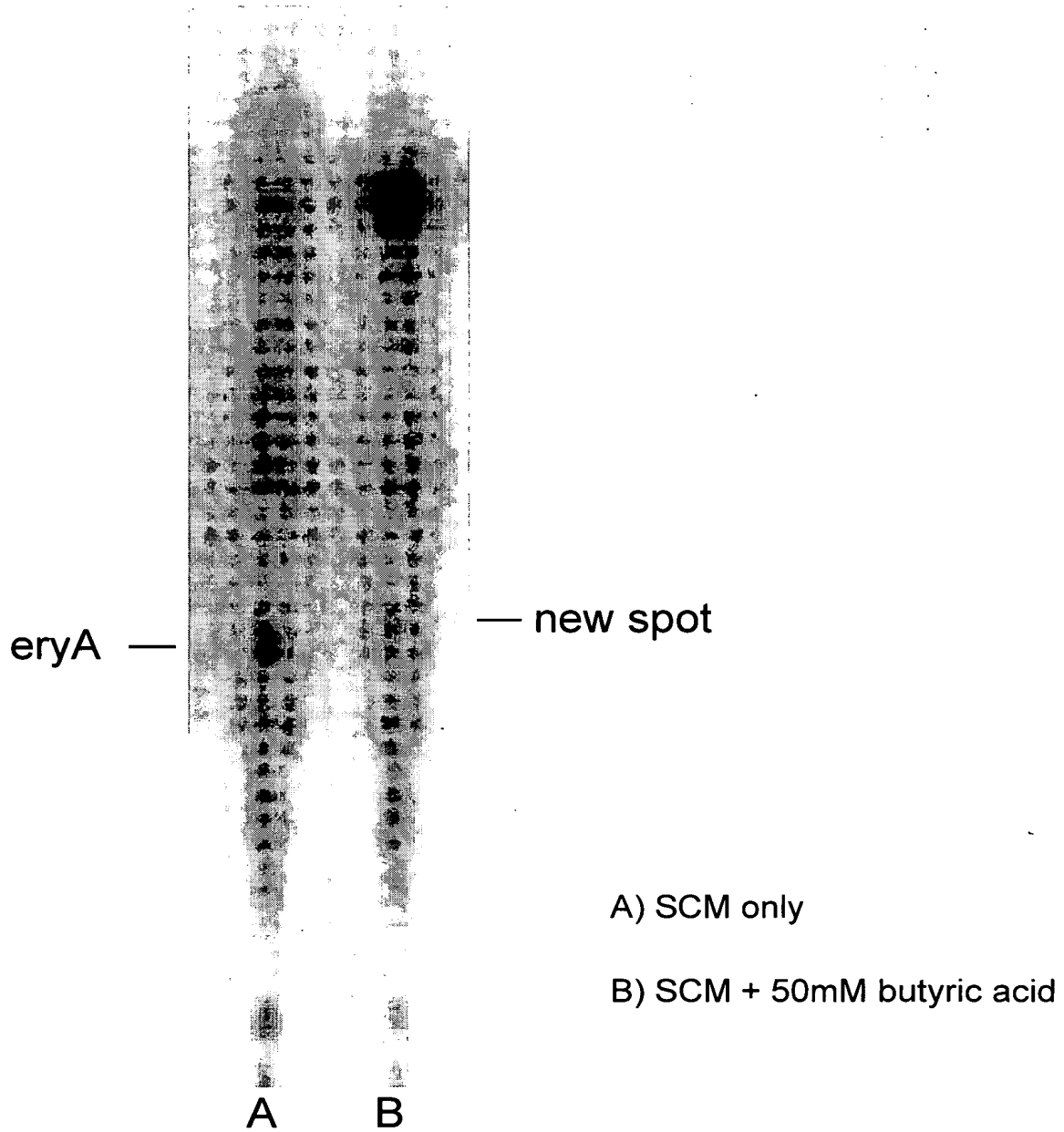


FIG. 39

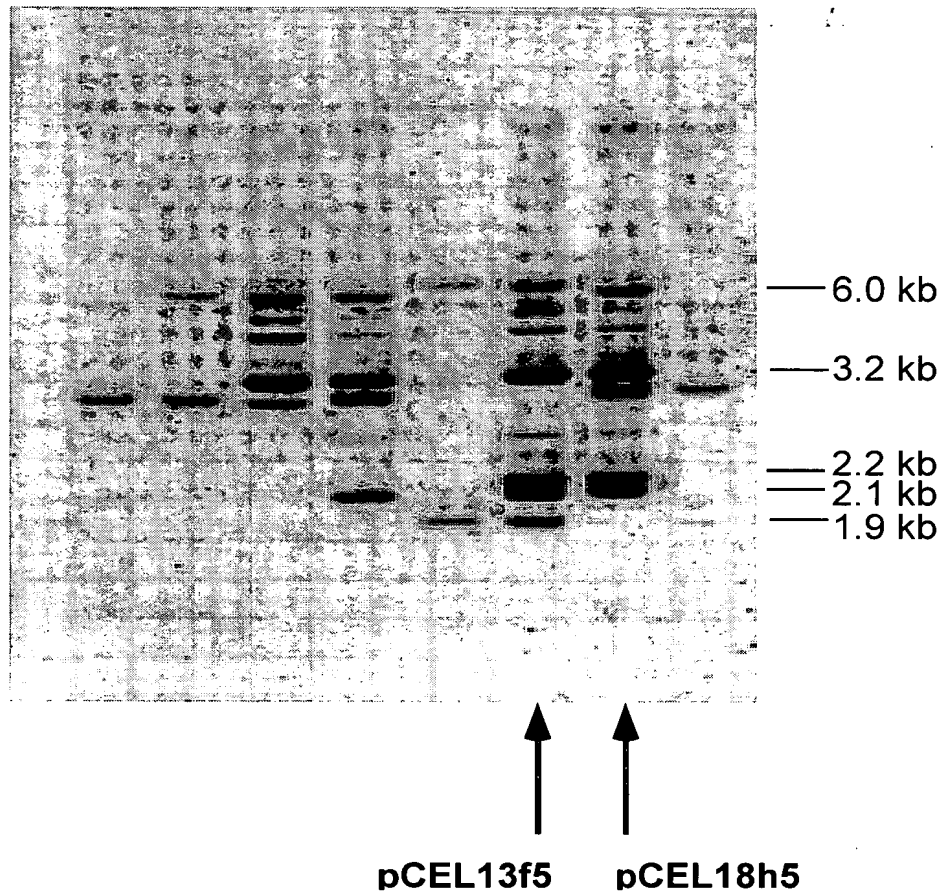


FIG. 40

CGCGCGCTGCCTTCGTCTTTCCCGGGCAGGGCGCCAGTGGGCCGGACTGGGAGCGCGG 60  
 R A P A F V F P G Q G A Q W A G L G A R 20  
 CTCCTCGCGGACTCCCCGTCTTCCGCGCCAGGGCCGAGGCATGCGCGCGGGCGCTGGAG 120  
 L L A D S P V F R A R A E A C A R A L E 40  
 CCTCACCTCGACTGGTCCGTCTCGACGTGCTGGCCGGCGCCCCGGGCACCCCTCCCATC 180  
 P H L D W S V L D V L A G A P G T P P I 60  
 GACCGGGCCGACGTGGTGCAGCCGGTGCTGTTACACAGATGGTCTCGCTGGCCGCCCTC 240  
 D R A D V V Q P V L F T T M V S L A A L 80  
 TGGGAGGCCACGGGGTCCGGCCGGCCGGTCTGGGCCACTCCCAGGGCGAGGTGGCC 300  
 W E A H G V R P A A V V G H S Q G E V A 100  
 GCGGCCTGCGTGGCCGGTCCCCTGTGCTGGACGACGTGCCCTGGTGATCGCCGGACGC 360  
 A A C V A G A L S L D D A A L V I A G R 120  
 AGCAGGCTGTGGGGGCGCTGGCCGGAACGGCGGGATGCTCGCGGTGATGGCTCCGGCC 420  
 S R L W G R L A G N G G M L A V M A P A 140  
 GAGCGATCCGTGAGCTGCTCGAACCATGCCCGCAGCGATTTCGGTGGCGCGGTCAAT 480  
 E R I R E L L E P W R Q R I S V A A V N 160  
 GGCCCCGCCTCGGTACCGTCTCCGGTGACCGCTCGCGCTGGAGGAGTTCGGCGCGCGG 540  
 G P A S V T V S G D A L A L E E F G A R 180  
 CTCTCCGCCGAGGGGTGCTGCGCTGGCCGCTGCCGGCGTCTGACTTCGCCGGCCACTCG 600  
 L S A E G V L R W P L P G V D F A G H S 200  
 CCGCAGGTGGAGGAGTTCC GC5CTGAGCTCCTGGACCTGCTCTCCGGCGTACGGCCGGC 660  
 P Q V E E F R A E L L D L L S G V R P A 220  
 CCTTCGCGGATACCTTTCTTCTCCACCGTGACGGCGGGTCTTGCGGCGGCGACCAGCTG 720  
 P S R I P F P S T V T A G P C G G D Q L 240  
 GACGGGGCGTACTGGTACCGCAACACGCGGAACCCGTGGAGTTCGACGCCACGGTCCGG 780  
 D G A Y W Y R N T R E P V E F D A T V R 260  
 GCGCTGCTGCGTGGGGCCATCACACGTTTCATCGAGTCCGTCCGCATCCGCTGCTCAAC 840  
 A L L R A G H H T F I E V G P H P L L N 280  
 GCCCGCATCGACGAGATCCGACGGACGAGGGGGTAGCGGCCACGGCCCTGCATACGCTC 900  
 A A I D E I A A D E G V A A T A L H T L 300  
 CAGCGGGGCGTGGCGGCCTTGACCGCGTGCGCAACGCGGTGGGCGCCGCTTTCGCGCAC 960  
 Q R G A G G L D R V R N A V G A A F A H 320  
 GGTGTCCGGTCCGACTGGAACGCCCTGTTGAGGGCACCGGTGCGCGCAGGGTGCCGCTT 1020  
 G V R V D W N A L F E G T G A R R V P L 340  
 CCCTCGTACGCCTTC 1035  
 P S Y A F 345

FIG. 41

## PCR OLIGOS:

N-TERMINAL OLIGO: 5' *Eco*RI Tag-<sup>*Avr*II</sup>  
 CCTAGGGTCGCCTTCGTCTTTCCCGGGCAGG-3'  
 GCGC CCT

ENGINEERED <i>Avr</i> II AND <i>Va</i> I CODON	HOMOLOGOUS REGION
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C-TERMINAL OLIGO: 5' *Bgl*II Tag-<sup>*Nsi*I</sup>  
 ATGCATACGAGGGAAGCGGCACCCTGC-3'  
 G G

ENGINEERED <i>Nsi</i> I	HOMOLOGOUS REGION
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## PCR CLONING:

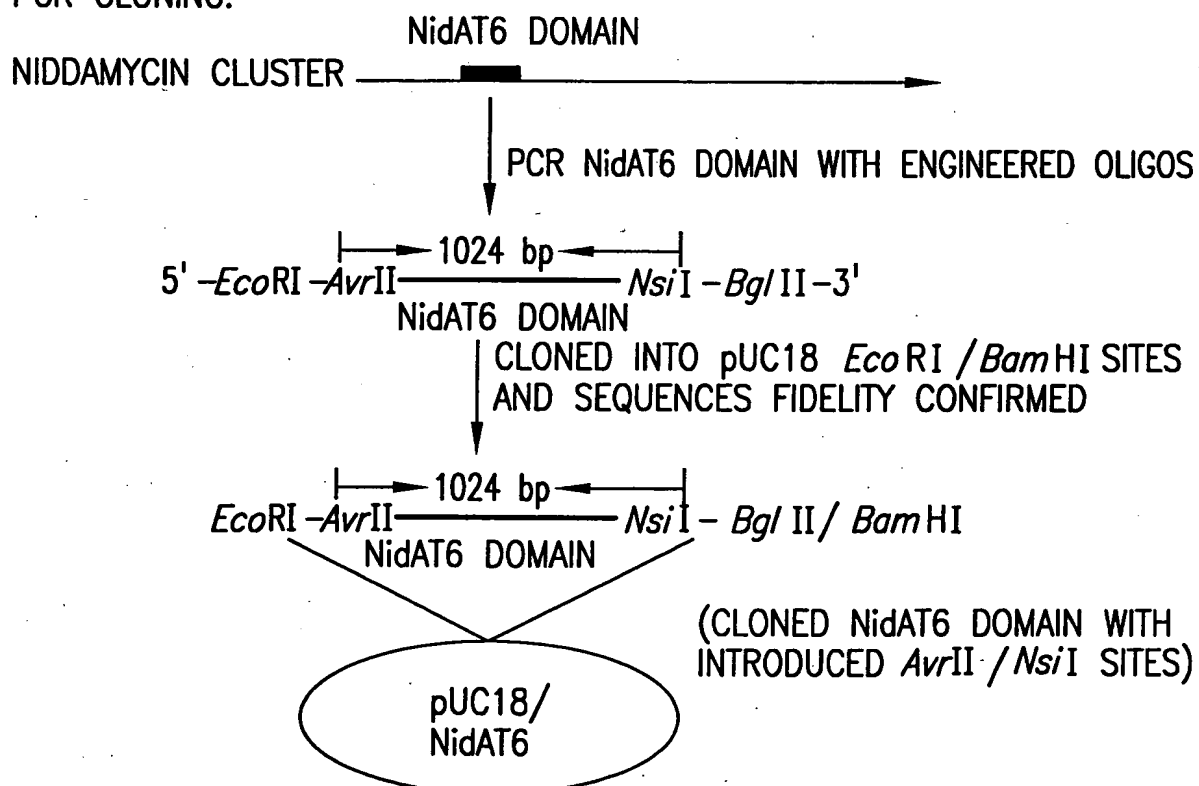


FIG.42

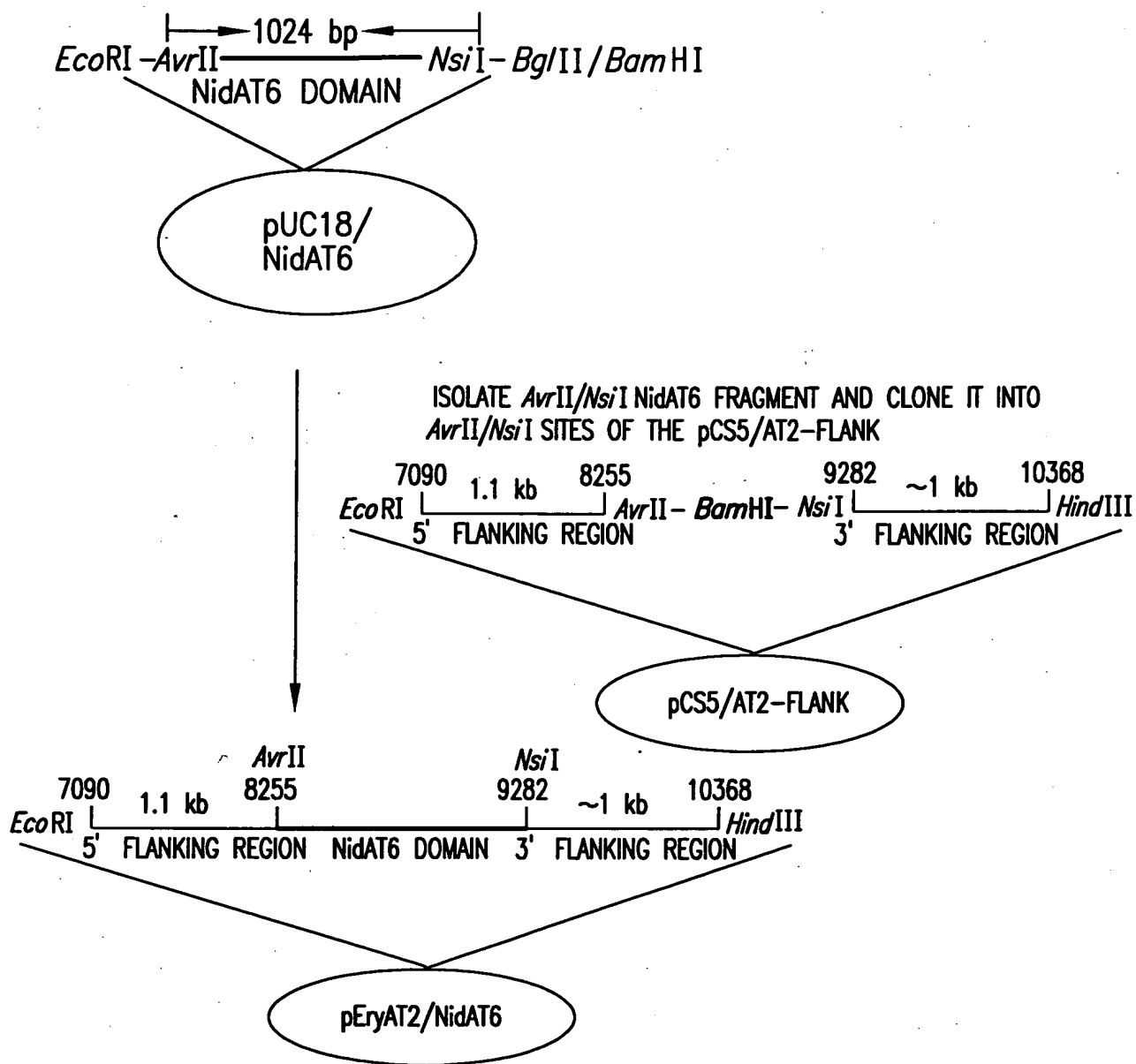


FIG.43